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EXPLORATORY POT EXPERIMENTS ON SENSITIVENESS OF DIFFERENT CROPS TO SODIUM: B. OATS

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INTRODUCTION

To investigate the sodium response of different crops, a series of simple pot experiments were made in 1943 according to the scheme set forth in a previous paper¹⁰). The present communication refers to two experiments with oats (variety "Mansholt III"), one of which was on a mixture of dusarit-sand and the other on soil. No analyses could be made on the plant material because this was lost at Wageningen during the war at the end of 1944. This communication is concluded by a discussion of the available literature as a further explanation of the importance of sodium for oats.

EXPERIMENTAL

A. Oats in glass pots on mixtures of dusarit-quartz sand.

Pots with 2.3 kg quartz sand + 100 g dusarit; water supply at 60% of the water-holding capacity.

Basic dressing: 540 mg $\text{CaHPO}_4 \cdot 2\text{aq.}$, 15 mg FeCl_3 , 50 mg $\text{MnSO}_4 \cdot 4\text{aq.}$, 50 mg $\text{CuSO}_4 \cdot 5\text{aq.}$, 5 mg borax.

Potassium: levels of 1, 3 and 8% K-dusarit; the mixture also contained 5% Mg-dusarit and was made up to 100% with Ca-dusarit. The pH of this mixture was 6.2.

Nitrogen: 300 mg N in the form of NaNO_3 or $\text{Ca(NO}_3)_2$, as follows: 100 mg at the beginning of the experiment and the remainder in two portions as top-dressing.

Pots in triplicate; 12 plants per pot.

During the vegetative period the plants with $\text{Ca(NO}_3)_2$ and 1% K-dusarit soon lagged behind in growth. Shortly after the last

topdressing these plants showed many yellow leaves and withering symptoms at the leaf tips. Later they recovered considerably, but the colour remained unhealthy and particularly in the chaff, was darker than that of normally matured plants. The group with NaNO_3 and 1% K was slightly below normal, but showed no special symptoms. In the pots with 3 and 8% K the $\text{Ca}(\text{NO}_3)_2$ plants temporarily showed a slightly more superior habit (broader and sturdier leaves) than those with NaNO_3 . It is noteworthy that the straw of the NaNO_3 plants at all K levels was more sturdy throughout the growing period, particularly at the base.

TABLE I

Yields of oats on dusarit-sand						
Dressing	grain in g dry matter per pot	straw in g dry matter per pot	1000- grain weight in g	straw length in cm	average weight of straw in g/cm	length of panicle in cm.
NaNO_3 — 1% K	14.2 ± 0.44	16.9 ± 0.35	25.0	87	0.194	12.8
„ — 3% K	17.0 ± 0.79	19.0 ± 0.68	29.7	92	0.206	14.0
„ — 8% K	18.6 ± 0.29	20.5 ± 0.25	32.0	97	0.211	13.6
$\text{Ca}(\text{NO}_3)_2$ — 1% K	12.2 ± 1.21	14.1 ± 0.62	27.4	(90)	0.166	12.4
„ — 3% K	16.3 ± 0.55	16.9 ± 0.26	29.2	92	0.184	13.2
„ — 8% K	16.0 ± 0.39	18.0 ± 0.24	28.8	90	0.200	13.7

B. Oats in glass pots on humic sandy soil.

The same soil was employed as was already described in ¹¹), page 40).

Pots with 2.07 kg of dry soil, maintained at 60% of the water holding capacity.

Basic dressing: 1.5 g $\text{Ca}(\text{OH})_2$, 540 mg $\text{CaHPO}_4 \cdot 2\text{aq.}$, 300 mg $\text{MgSO}_4 \cdot 7\text{aq.}$, 15 mg FeCl_3 , 50 mg $\text{MnSO}_4 \cdot 4\text{aq.}$, 50 mg $\text{CuSO}_4 \cdot 5\text{aq.}$, 5 mg borax.

Potassium: levels of 0; 300 and 700 mg KCl of which 175 and 400 mg respectively were applied as top-dressing.

Nitrogen: 300 mg N in the form of Chilean nitrate or nitrate of lime as follows: 100 mg at the beginning of the experiment and the remainder in two portions as top dressing.

Pots in triplicate; 12 plants per pot.

On soil the development of the plants was a little sturdier than on dusarit-sand. There was no evidence of damage from a K deficiency, although the $\text{Ca}(\text{NO}_3)_2$ plants without K lagged behind in growth. With the K dressing the $\text{Ca}(\text{NO}_3)_2$ plants also showed initially a somewhat heavier leaf development than the NaNO_3 plants, but the differences were minimal at the final stage.

TABLE II

Yields of oats on soil						
Dressing	grain in g dry matter per pot	straw in g dry matter per pot	1000- grain weight in g	straw length in cm	average weight of straw in g/cm	length of panicle in cm
chilean nitrate						
without KCl . .	20.3 $\pm$ 0.40	18.2 $\pm$ 0.12	31.5	86	0.211	13.8
300 mg KCl . .	20.6 $\pm$ 0.61	19.7 $\pm$ 0.73	31.2	88	0.224	14.1
700 mg KCl . .	20.8 $\pm$ 0.32	19.6 $\pm$ 0.17	31.3	89	0.220	14.6
nitrate of lime						
without KCl . .	18.4 $\pm$ 0.09	17.9 $\pm$ 0.35	31.1	90	0.199	13.3
300 mg KCl . .	(19.2) $\pm$ 1.48	19.7 $\pm$ 0.48	31.8	91	0.216	14.0
700 mg KCl . .	21.4 $\pm$ 0.20	20.3 $\pm$ 0.17	32.5	90	0.226	14.8

DISCUSSION

Although oats are not described as outstanding with regard to their capacity to take up sodium^{3) 7) 12)}, it has been shown that in oat straw (vegetative stage) K and Na can replace each other equivalently within wide limits^{11) 13)}; in the case of a low supply of potassium therefore the oat plant can take up considerable quantities of sodium.

There exists a fairly extensive literature on sodium as a nutrient for oats which — apart from papers on salt resistance — almost unanimously describes this element as beneficial to oats with the power to partially replace potassium. Only one author (Stahl Schroeder¹⁶⁾) strongly opposes the conception of sodium as a physiologically active element. Nevertheless, it is the older authors in particular who are unwilling to ascribe to sodium anything more than a substituting function, thus in reality considering sodium to be a non-essential nutrient. On further scrutinizing the available literature so as to arrive at a more critical appraisal of the importance of sodium, one is inclined to conclude that the way in which the sodium problem was stated in the past was erroneous.

The oats experiments are a typical illustration of the fact that the older investigations, still frequently referred to as evidence for the non-essentiality of sodium as a plant nutrient, are not at all so negative when the problem is approached from a different angle. Owing to the fact that sodium has not appeared to be an absolute substitute for potassium the possibility that sodium could have an independent physiological function in the plant has been disregarded. As a result, inadequate attention was paid to the

question whether, in presence of sufficient potassium, sodium still has an effect of its own.

For a better classification of the data which can be used to give a fairly reliable answer, we must first consider the theoretical possibilities of the yield curves.

In experiments with increasing doses of potassium and a fixed dose of sodium it is possible to distinguish, in principle, two types of yield curves:

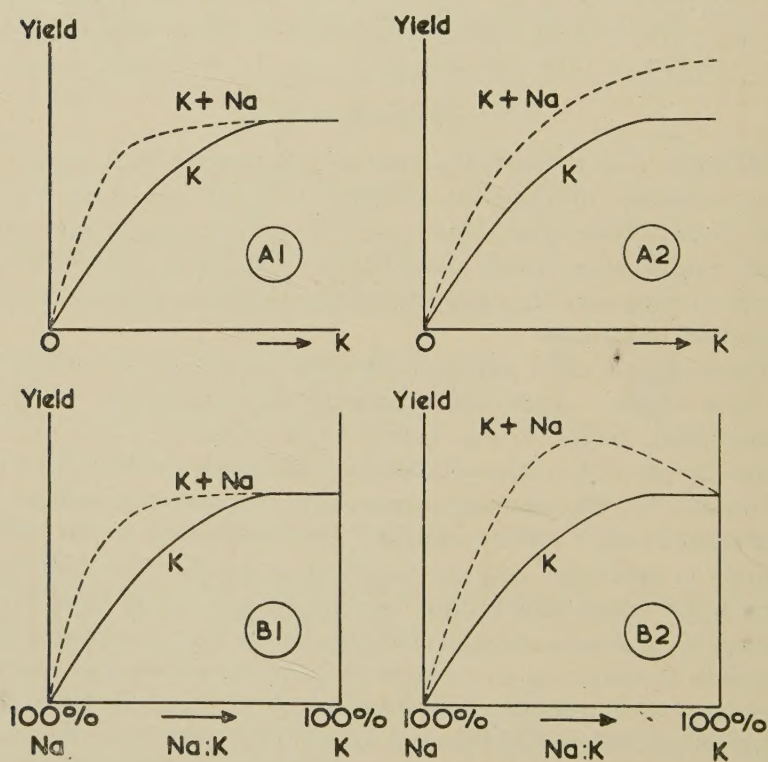


Fig. 1. In experiments with increasing doses of potassium with the addition of a constant amount of sodium a trend of the yield curves, as under A 1, indicates a replacing action of sodium, without a specific function of its own. If the yield curves run as under A 2, an independent function may be ascribed to the sodium.

In experiments with a constant number of equivalents of potassium and sodium, but with varying ratios of these two elements, B 1 represents the case of partial replacement of potassium by sodium, while B 2 indicates an independent action of sodium (necessary for maximum production).

Type A1 in fig. 1 implies that in case of inadequate potassium the application of sodium has a yield-increasing effect which however, declines at the higher K doses and disappears at the maximum of the K effect. This type of curve indicates partial replacement of potassium by sodium, but excludes an essential function of sodium on its own. On the other hand, the trend of the curves A2 points to a function of sodium that is not dependent on K, because even at the optimal supply of K there is a yield-increasing effect due to Na.

Apart from experiments of the above type, there are experiments in which the total number of equivalents of K and Na were kept constant in the nutrient medium, but with numerous variations in their mutual ratios. Fig. B1 and B2 refer to these types of experiment. The lower curves show the effect of K in the absence of Na and therefore correspond to the K curves in fig. A1 and A2. Case B1 represents the theoretical case in which Na does have a replacing effect, but no effect of its own. Case B2 shows, finally, that for maximum yields one cannot confine oneself to K alone, since a certain amount of Na is essential for maximum yields.

The following table summarizes the results of the investigations, published so far, according to the behaviour of the yield curves. The trends as in cases A1 or B1 have been indicated with + in the column "K/Na replacement". If the results were identical with case A2 or with B2 a corresponding mark has been made in the column "independent effect of sodium".

TABLE III

Year	Author	Type of experiment	K/Na replacement	Independent effect of sodium
1868	Wolff ¹⁸⁾	B. water cultures		+
1891	Atterberg ¹⁾	B. sand-water cultures	+	
1898	Hellriegel, c.s. ⁵⁾	B. sand cultures		+
1902	Wilfahrt and Wimmer ¹⁷⁾	A. sand cultures		+
1904	Damseaux ⁴⁾	B. sand cultures		+
1918	Mitscherlich ¹⁴⁾	A. pot experiments		(+)
1920	Pfeiffer and Rippel ¹⁵⁾	B. sand cultures	+ *)	
1936	Boguslawski ²⁾	A. pot expt. loamy/sandy soil		+
1945	Holt and Volk ⁶⁾	A. sand cultures		+
1949	Larson ⁹⁾	A. pot expt. loamy soils		+
....	present paper	A. pot expt. sand-dusarit		+
....	present paper	A. pot expt. sandy soil	+ *)	

*) In these experiments the medium itself contained considerable amounts of sodium

For a correct appraisal of the last column it should be noted that, although all the particular authors themselves consider their highest K dose to be ample, a maximum K effect was not achieved in all experiments. The trend of the curves is, however, such that the maximum can be estimated, so that on the basis hereof the Na effect at optimal K effect can be inferred.

In two instances ⁶⁾ and ¹⁷⁾ the Na addition was considerably in excess of K. The experiments of Mitscherlich cause some difficulty, as points are missing in an important curve; the trial scheme moreover was complicated since K was added as KNO_3 , while the chlorine concentration was kept constant by the application of varying amounts of NaCl and NH_4Cl . According to the trend of the curves an independent Na effect is very likely, though, at a very high K dose, probably exceeding by far the maximum dose needed, it amounted at most to 6%.

Larson ⁹⁾ carried out three experiments of which the first (on Harpster silt loam — F 219) was very consistent and showed an independent sodium effect. The second experiment (on Carrington Loam — F 361) showed irregular grain yields but total yields indicated a positive sodium effect at all K levels. His third experiment (Harpster Silt Loam — F 362) gave irregular data for a single sodium dose but quite convincing results with a double dose of sodium.

The execution of the oldest experiment ¹⁸⁾ was still very defective.

In the experiments which only admit of a certain K/Na replacement it appears to us that only that of Atterberg is convincing; this investigator replaced K in his nutrient solutions by equivalent amounts of Na and Ca respectively. Unfortunately, the documentation of this experiment is inadequate. With regard to the investigation by Pfeiffer and Rippel ¹⁵⁾ it may be observed that the sulphate form with which they worked is probably the least favourable for experiments on the effect of K and Na, while the large amounts of sodium contained in the quartz sand employed (vide the relative analyses) render incertain a negative conclusion as to the essentiality of this element. Jakob ⁸⁾ also pointed out how difficult it is to make a reliable experiment owing to the not unappreciable quantities of K and Na taken up by the crop from the sand employed. It is evident that, if the

medium itself contains appreciable amounts of Na, the yield curves of fig. 1 are influenced by it and that in particular the basic curve for K is no longer a true K line. It should be mentioned here, that also in Larson's second and third experiments high contents of sodium are stated for the straw.

Our own experiments present an example of the fact that, depending entirely on the Na content of the medium, one may arrive at results which suggest either a more essential function of Na or at the most a certain K-replacing value. With the complete exclusion of Na from the medium of the dusarit experiment, the NaNO_3 dressing with increasing K doses gave yields of 20; 12.5 and 14% straw (all significant) and 16.5; 4 and 16% grain (significant at the highest K dose) over those obtained with the $\text{Ca}(\text{NO}_3)_2$ dressing. As the highest K dose with $\text{Ca}(\text{NO}_3)_2$ no longer produced an increase in yield, the positive effect of NaNO_3 points unmistakably to an independent function of Na.

In the pot experiment with soil the increased yields with Chilean nitrate of soda at rising K levels were 2; 0 and —5% straw and 10.5; 7 and —3% grain (only the surplus yield on omitting K dressing significant). The soil in question, although emanating from control plots of a potash field experiment, still appeared to have a fair K content and a distinctly high Na content (the soil added 200 mg exchangeable Na per pot). In these circumstances the Na dressing only proved advantageous with a low K dose or none at all. For an appraisal of the pure Na effect this soil experiment is, of course, not very suitable.

Although the conclusions drawn by the author from the above review, differ from prevailing general conceptions as to the function of sodium, it is in his opinion more appropriate to ascribe to sodium an independent effect in the nutrition of oats than merely a K-replacing effect. Such a physiological effect may not yet have manifested itself by specific deficiency symptoms, but in any case it appears to be essential for optimal yields. It is very desirable to gather more effective evidence on this by experiments which should duly take into account the particular character of the element sodium.

As a last point we would stress that it sometimes appears that Na favours vegetative development rather than the formation of grain, such as we have also experienced in field experiments ¹¹).

This lack of agreement with the pot trials described above may be explained by a difference in the method of fertilizer application. In the said field experiments, all nitrogen was added in one dose on sowing, while the N application in the pot experiment was divided over 3 doses (at the beginning, then 3 and 6 weeks after sowing). It is possible that an early NaNO_3 application causes the fixation of a larger amount of nitrogen in the straw owing to more abundant vegetative development, thus leaving less N available for grain formation (on N-poor soils). Full benefit from the Na-effect (for grain also) must try to be obtained either by subdividing the N dose (especially if added in the form of NaNO_3) or by an extra N top dressing at the earing stage.

SUMMARY

Two pot experiments with oats are described: 1. on an artificial soil complex (sand-dusarit mixture) and 2. on a humic sandy soil, in which the Na effect of nitrate of soda was studied by comparison with calcium nitrate. On the Na-free sand-dusarit mixture a positive Na effect on grain and straw was obtained at all K levels. On the other hand, the Na effect in the soil experiment was confined to the pots without K dressing. The soil itself however, had an appreciable K content and a high Na content.

A further study of the literature on pot experiments with oats leads to the conclusion that the Na effect was incorrectly interpreted in the past as a K-replacing effect, whereas the results point mostly to an independent function of Na by itself (positive yield effects in the presence of sufficient potassium). Out of 12 available examples, only 3 turn out to be in favour of a mere K-replacing action. Moreover, 2 of them cannot be called conclusive, because of unmistakable presence of fairly large amounts of Na in the growing medium.

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COMPARATIVE EFFECTS OF SODIUM NITRATE AND CALCIUM NITRATE ON THE POTATO

by ONORATO VERONA and ANTONIO BENVENUTI

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Investigations on the potato have been included within the framework of researches carried out by this Institute on the influence of sodium on plants, in connection with their growth, health, and, finally, yield ^{5), 6)}. It was thought that the potato would respond to this element as plants with high potassium content usually do. Actually we know that sodium substitutes for potassium, even if only to a small extent, or at any rate it contributes to a greater mobility of the same.

We were interested in investigating the effects of sodium on this plant particularly as some research workers state that this element appears to be assimilated with difficulty by the potato. It is true, however, that even if the quantity of sodium in the tubers is small, a considerable quantity of it is to be found in stalk and leaves ^{1) *)}.

Experiments in this connection had been previously carried out by Metz ³⁾ and Süchting ⁴⁾, demonstrating respectively with good results the action of both sulphate and sodium carbonate; later Maschhaupt ²⁾ also obtained good results with sodium nitrate applications.

The experiments referred to herein have been carried out on plots in the Experimental Field of the Institute and in a field of the Experimental Farm attached to the Institute of Agronomy. We shall discuss these separately.

*) These Authors give the following data:

% in the ash:	{	<i>tubers</i>		<i>stalks and leaves</i>	
		Na	0.0655		0.5940
	{	K	47.71		30.11
	{	K/Na	729		50.7

I. EXPERIMENTS ON PLOTS IN THE EXPERIMENTAL FIELD
OF THE INSTITUTE

— Plots of m. 1.50 × 1.50 (sq.m. 3)

A	D	C	F	N ↗
B	E	B	E	
C	F	A	D	

- Soil: fallow
- Basic fertilizer: farmyard manure 300 ql per ha
- Potassium and Nitrogen: as follows:
 - A) no Potassium; Sodium nitrate: 120 kg/ha of N
 - B) do Calcium nitrate: do
 - C) K₂O: 125 kg/ha; Sodium nitrate: do
 - D) do Calcium nitrate: do
 - E) K₂O: 250 kg/ha; Sodium nitrate: do
 - F) do Calcium nitrate: do

Potassium was supplied before sowing in the form of potassic sulphate; Nitrogen was supplied in two portions, one half of the total 30 days from sowing, the other half ten days later.

The sowing was carried out on April 3rd by using entire tubers of the variety Jubel X Tonda di Berlino, selection 61-1-4 (Avanzi).

Soil analysis: pH.	7.5
loss on ignition %.	4.75
CaCO ₃ %	10.86
Nitrate N (mg per 100 g)	10.80
Ammonia N (mg per 100 g)	3.50
P ₂ O ₅ soluble in water (mg per 100 g)	19.30
K ₂ O } soluble in citric acid 1%.	19.—
Na ₂ O } (mg per 100 g)	34.—
CaO% } soluble in hydrochloric acid.	7.26
MgO% }	1.07

Growth occurred normally and no parasitic attacks of any nature nor virus infections were noted. Harvesting of the tubers was carried out on July 20th and the following data were obtained:

Plots	Average data per plot		Average data per plant	
	Weight of product kg	Number of tubers	Weight of tubers kg	Number of tubers
A	5.66	138	0.377	9.2
B	4.63	127	0.308	8.5
C	5.51	134	0.367	8.9
D	4.56	130	0.304	8.6
E	4.82	126	0.321	8.4
F	5.14	125	0.342	8.3

II. EXPERIMENTS ON PLOTS IN THE EXPERIMENTAL FARM OF THE INSTITUTE OF AGRONOMY

Plots of m. 2.50×2.50 (sq.m. 6.25)

A	B	C	D	E	F	N ↗
D	E	F	A	B	C	
A	B	C	D	E	F	
D	E	F	A	B	C	

Previous crop: turnips and oats for green fodder, following wheat.
Basic fertilizer farmyard manure 300 ql per ha, applied to turnips and oats.

Potassium and nitrogen, as in the plot experiment n° I.

The sowing was carried out with entire tubers on April 16th with the same variety employed in plot experiment n° I.

Soil analysis:

pH.	7.6
loss on ignition %.	3.92
CaCO ₃ %	9.80
Nitrate N (mg per 100 g)	5.90
Ammonia N (mg per 100 g)	1.70
P ₂ O ₅ soluble in water (mg per 100 g)	tr.
K ₂ O } soluble in citric acid 1%	15.—
Na ₂ O } (mg per 100 g)	32.—
CaO% } soluble in hydrochloric acid	6.85
MgO% }	1.93

During these trials also growth progressed normally since no parasitic attacks were to be noted. At the harvest, which was carried out on July 30th, the following data were obtained:

Plots	Average data per plot		Average data per plant	
	Weight of product kg	Number of tubers	Weight of tubers kg	Number of tubers
A	15.40	339	0.481	10.5
B	14.90	369	0.465	11.5
C	16.40	330	0.512	10.3
D	13.90	323	0.432	10.0
E	17.40	409	0.543	17.4
F	17.—	363	0.515	11.—

DISCUSSION ON THE RESULTS

Growth, as has been mentioned already, progressed normally everywhere. It should be added, however, that in the plots where so-

dium nitrate had been applied, the plants showed, in comparison with the others, a better growth and more intense colour. Moreover, in the plots to which potassium was not supplied, slight symptoms of K-deficiency were noticed in the presence of calcium nitrate, but not in the presence of sodium nitrate. This suggests, evidently, a larger uptake of potassic salts by the plants which had been fertilized with sodium nitrate, such as is also indicated by the results of the following analysis:

Analysis of the ash of the stalks and leaves of potatoes fertilized with sodium nitrate and calcium nitrate from plots which had not been fertilized with potassic salts.		
Ash % of dry matter:	In the presence of	
	NaNO ₃	Ca(NO ₃) ₂
	18.25	17.45
in the ash:		
Na ₂ O %	3.34	3.08
K ₂ O %	23.88	20.84
CaO %	28.12	26.45
MgO %	2.27	2.24

The same data indicate still more, that in the presence of sodium nitrate a greater total absorption of salts takes place, along with an increased absorption of calcium. It should be noted that the soil on which the experiments were carried out had a pH 7.5-7.6 and that more significant results could perhaps have been obtained if the soil had had a higher hydrogen-ion concentration.

If we consider the vegetation data, we note that the harvest from the plots fertilized with sodium nitrate was always more or less higher than from those where calcium nitrate had been supplied. This may be reasonably explained in terms of a greater uptake of salts, particularly of potassium, (which is very important in the synthesis of carbohydrates), in relation, perhaps, to the presence of sodium.

SUMMARY

Comparative trials with sodium nitrate and calcium nitrate on the potato indicate a certain superiority of the former, probably due to a greater utilization of potassium and hence improved carbohydrate metabolism.

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“ZINNIA MOSAIC”, A NEW VIRUS

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INTRODUCTION

During the autumn of 1951 in a public garden of Madrid, all the plants in a plantation of *Zinnia elegans* L. showed a strong mosaic, stunting and, in few cases, necrosis, all symptoms very characteristic of a virus disease. Since all the plants showed these symptoms there was however also the possibility of a genetic cause.

Inoculations with straight sap, from zinnia showing strong mosaic and necrosis, were made to the leaves of *Nicotiana Tabacum*, *N. glutinosa* and turnip using carborundum powder as abrasive.

In *N. Tabacum* necrotic lesions appeared all over the inoculated leaves in four days. The lesions enlarged and became confluent after two days and caused the death of the inoculated leaves a few days later.

Fifteen days after the inoculation, the first symptoms of a systemic infection on the younger leaves of *N. Tabacum* and *N. glutinosa* appeared, developing typical symptoms which will be described later. The turnip plants remained healthy.

Once convinced that it was a virus disease, different plants were inoculated and aphid transmission of the disease and the physical properties of the infected sap were studied.

The results of these and other experiments showed that an apparently undescribed virus was concerned and a short, preliminary account of it is given in the present paper.

DESCRIPTION OF THE VIRUS

Transmission

The virus is easily sap-transmissible and usually gives more than 70% infection with straight sap inoculation.

The insect vectors, if any, are not known. Transmission tests with the aphides *Mizus persicae* Sultz, *Aphis fabae* (found on the zinnia plants) and two other aphids, which were not named, have proved negative.

Thermal inactivation

The virus is inactivated at 55°C in 10 minutes but not at 50°C.

Dilution end-point.

Infections have been obtained at a dilution of 1/10 but not at 1/100.

Host range and symptomatology

Zinnia Elegans. The principal characteristic is a strong mosaic, stunting, decrease of the number of the flowers and breaking of the colour.

Some plants showed necrosis also, but it was proved that necrosis was produced by another virus, which causes also the primary necrotic lesions on tobacco. These few plants were infected with both viruses. The virus causing necrosis could be eliminated by one passage through *N. Tabacum*.

Nicotiana Tabacum var. White Burley. The symptoms in this plant are rather severe; they start in 15–35 days (depending on the temperature) after inoculation of the leaves with undiluted infected sap using carborundum as abrasive, as a clearing of the veins in the younger leaves. This is followed by a mottle and strong mosaic and in a short time they begin to become deformed. Sometimes the laminae become so reduced that the leaves consist of little more than the mid-rib (plate 4).

Nicotiana glutinosa. In this plant the symptoms appear a few days earlier than in *N. Tabacum* as a strong mosaic, similar to that produced by cucumber mosaic in the same plant.

The leaves are somewhat smaller than the normal ones.

Non-susceptible plants

Three series of the following plants have been inoculated with the virus three times in successive periods, without success:

Nicotiana rustica

Lycopersicum esculentum var. Klondine red.

Datura Stramonium

Cucurbita Pepo

Vicia Faba



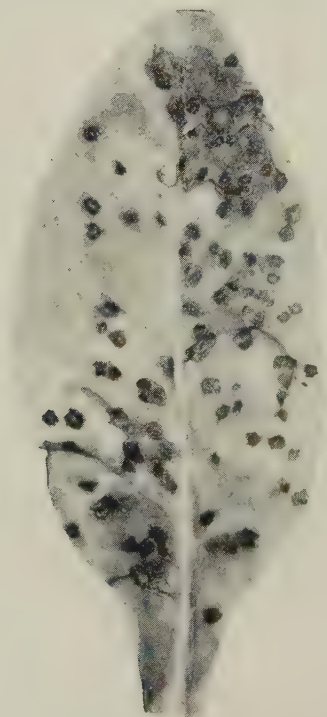
a. Plant of zinnia infected, showing mosaic in its leaves.



b. Some leaves of zinnia showing strong mosaic.



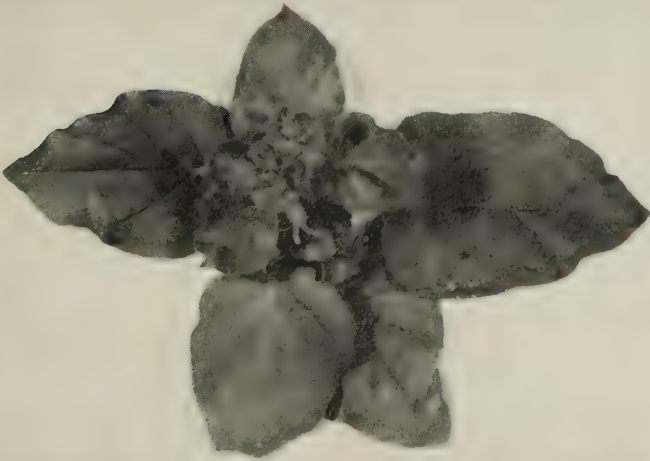
a. Flower from *Z. elegans* infected showing breaking of the colour (white stipes on the red, normal colour).



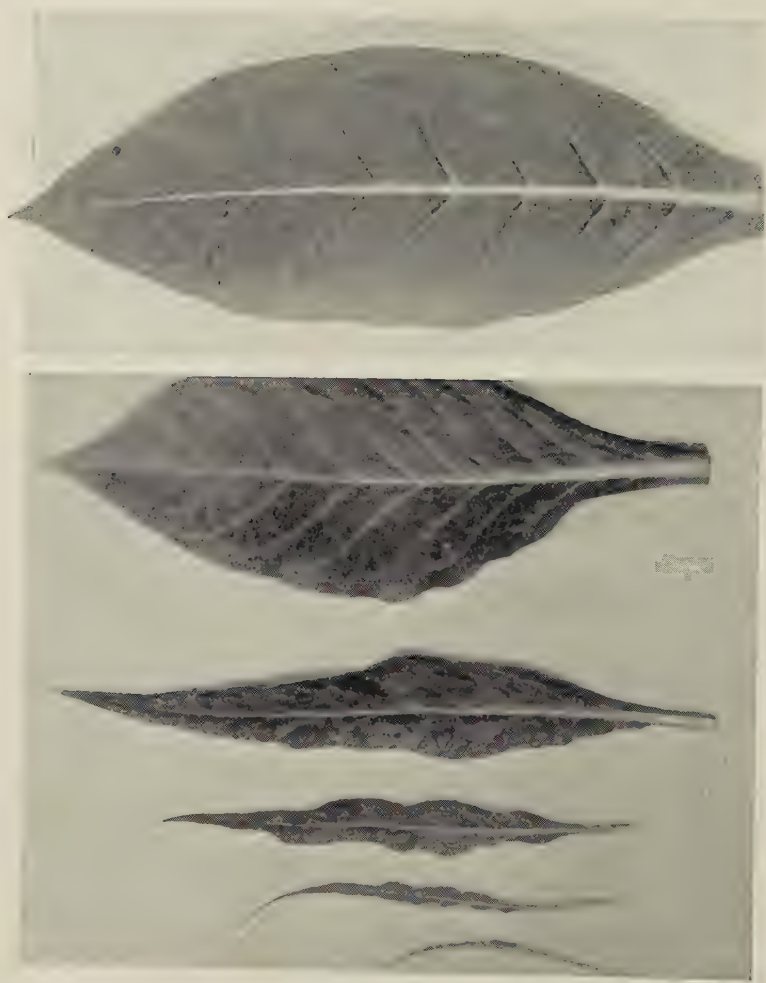
b. Necrotic lesions on *Nicotiana Tabacum* var. White Burley.



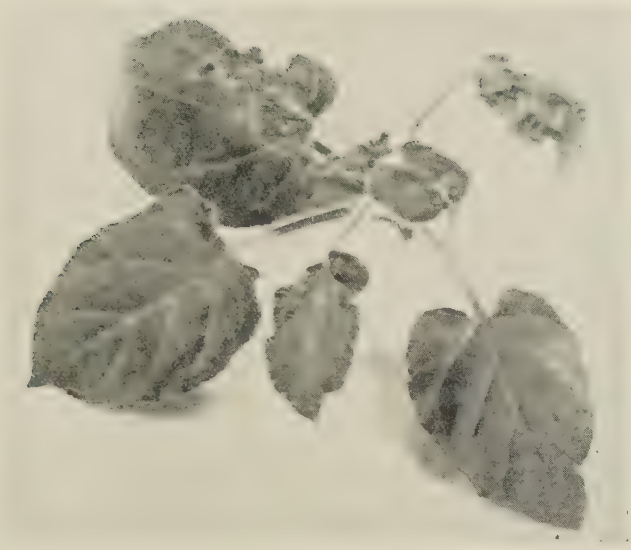
a. Systemic infection in *Nicotiana glauca* showing mosaic and deformation of the younger leaves. The older leaves remain normal.



b. Systemic infection in *Nicotiana glauca* showing mosaic and malformation in the younger leaves.



Leaves of *N. Tabacum*. The one at the right healthy; the other infected showing mosaic and a progressive reduction of the laminae.



a. *Nicotiana glutinosa* infected with zinnia mosaic virus (the younger leaves are stunted and show mosaic).



b. Two leaves of *Zinnia elegans* infected with zinnia mosaic virus. Photograph obtained by laying the leaves on photographic paper. They show a strong mosaic.

TABLE I

Viruses	Transmission		Phys. prop.		Test plants						
	In-sects	Thermal inactivation °C	Dilution end-point	Zinnia	N. glauca	N. Tabacum	Datura Stramonium	Tomato	Brassica ch.	Cucumber	Vigna sinensis
Definium Virus 1	—	—	—	+	—	+	—	+	—	+	—
Sugar beet curly top Beta V. 1	—	75-80	1/20,000	+	—	+	+	+	+	+	—
Cucumber mosaic	—	—	—	—	—	—	—	—	—	—	—
Cucumis virus 1	—	60-70	1/10,000	+	+	—	—	+	—	+	—
Californian Aster Yellows	—	—	—	—	—	—	—	—	—	—	—
Callistepus 1 A	—	—	—	—	—	—	—	+	—	—	—
Tobacco leaf curl	—	—	—	—	—	—	—	—	—	—	—
Nicotiana virus 10	—	—	—	+	—	—	—	—	—	—	—
Tobacco ringspot	—	—	—	—	—	—	—	—	—	—	—
Nicotiana virus 13	—	60	1/1,000	+	+	+	+	+	—	+	+
Tomato spotted wilt	—	—	1/10,000	—	—	—	—	—	—	—	—
Lycopersicum virus 3	—	42	1/100,000	+	+	+	+	+	+	—	—
Marmor brassicae	—	—	—	—	—	—	—	—	—	—	—
Turnip mosaic virus	—	54	1/1,000	+	+	+	—	—	+	—	—
Marmor medicaginis	—	—	—	—	—	—	—	—	—	—	—
Alfalfa mosaic virus	—	65-70	—	+	—	—	—	+	—	+	—
Zinnia mosaic virus	—?	55	1/100	+	+	+	—	—	—	—	—

Phaseolus vulgaris
Vigna sinensis
Vigna sexquipedalis
Brassica Rapa
Brassica chinensis.

In table I the properties of the virus being studied are compared with those of nine other well known viruses which infect *Zinnia elegans* L.

DISCUSSION

The properties of the virus being studied here compared with those from nine viruses known to infect *Zinnia elegans* L. (7 of them are described in the Text book of Plant Virus Diseases by K. M. Smith and the other two in the Bergey's manual of Determinative Bacteriology 6a ed.). It is evident that there are striking differences between those 9 viruses and the present one from zinnia.

In the first place, the Californian aster yellows and Tobacco leaf curl viruses can be eliminated as those viruses are not sap-transmissible. In the second place those viruses which are transmissible by aphids and which can infect some of the test plants not susceptible of infection with the virus from zinnia can be eliminated. These are: cucumber mosaic virus, marmor brassicae and marmor medicaginis.

The last 4 viruses: delphinium virus 1, sugar beet curly top, tobacco ringspot and tomato spotted wild, though not transmissible by aphids and transmissible by sap inoculation, can be differentiated easily from the present virus, because they infect some of the test plants not susceptible to infection by the virus from zinnia and also, because they exhibit different symptoms on plants also susceptible to infection with the virus under investigation.

The properties of several viruses, with similar symptomatology on *N. Tabacum* and *N. glutinosa* plants as the present virus, but not known to infect *Zinnia elegans*, have been investigated but none of them has been found to be identical with it.

The properties of the present virus are sufficient to make possible a clear differentiation from the other known viruses; therefore, we are most probably dealing with a new virus. Since it has been

isolated from *Zinnia elegans* and since its principal symptoms are a strong mosaic in all the plants tested, the name of "*Zinnia Mosaic Virus*" is proposed.

The occurrence of complete infection of a population of zinnia in the field and even in some seedlings proves that the virus is easily transmitted; more detailed investigations on the seed, soil and insect transmission, will be necessary and this, as well as the serology and cross-immunity relations of the zinnia mosaic virus, will be the subject of another paper.

SUMMARY

Some properties (host range, dilution end-point, temperature of inactivation, transmission and symptomatology) of a virus isolated from *Zinnia elegans* L. are described.

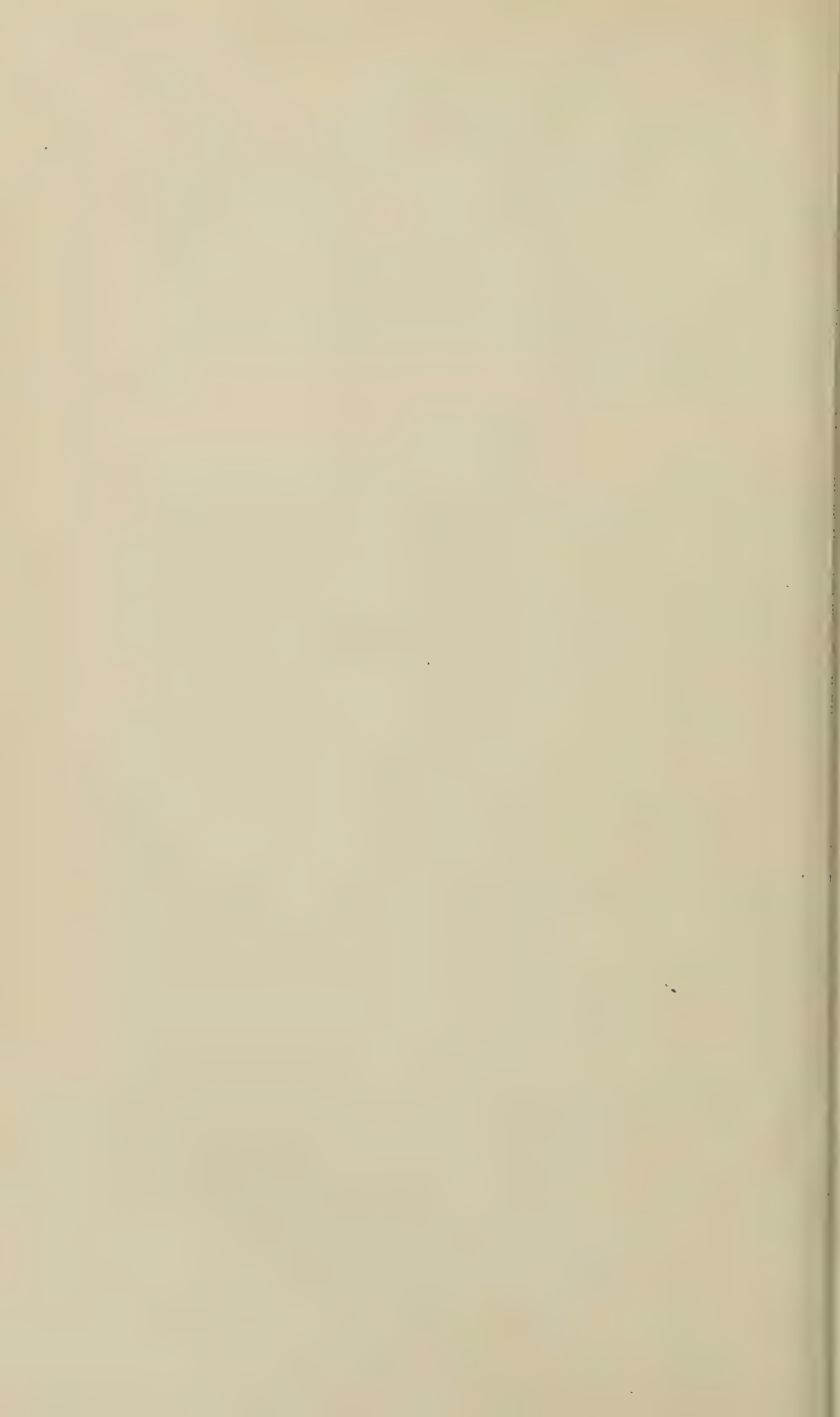
These properties have been compared with those from nine viruses already well known, infecting *Zinnia elegans*, and with other viruses related in some way to with the virus being studied.

The host range, transmission and physical properties of the virus from zinnia differ from those of the other viruses, which suggests that it is a virus not yet described and the name "*Zinnia Mosaic Virus*" is proposed.

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THE DONNAN-PRINCIPLE IN THE IONIC RELATIONS OF PLANT ROOTS

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INTRODUCTION

Since 1911 when Donnan^{1,2} first put forward his theory of membrane equilibria, the principle of the Donnan equilibrium has been applied to many biological problems. The Donnan equilibrium in its simplest form may be considered to be composed of two solutions or phases separated by a selectively permeable membrane. One of the solutions, the *i*-phase, contains indiffusible ions which are unable to migrate through the membrane to the other solution, the *o*-phase.

Consider an *i*-phase containing in addition to diffusible cations B^+ and diffusible anions A^- , a concentration of A eq. l. of indiffusible anions Z^- . The ions B^+ and A^- can move freely into the *o*-phase. Let their concentration in the *o*-phase at equilibrium be c . From thermodynamics it is known that in the equilibrium state the product of the concentrations of the ions B^+ and A^- in the *i*-phase is equal to the product of their concentrations in the *o*-phase. If the concentration of A^- in the *i*-phase is x , the concentration of B^+ must, in order that the electrical charges of anions and cations may balance, be $(A + x)$, so that

$$c \times c = (A + x)x.$$

It follows that the concentration of A^- in the *i*-phase will be less than in the *o*-phase ($x < c$), whereas there is a greater concentration of B^+ -ions in the *i*-phase than in the *o*-phase ($A + x > c$). The indiffusible ions thus bring about an increased concentration of ions of opposite charge. It can be demonstrated that in the case of more complex systems, containing a greater variety of ions, there

will also be a greater concentration of oppositely charged ions in the i-phase, irrespective of their type or valency. The ratio of the ionic concentration in the i-phase to the ionic concentration in the o-phase is greater the higher the valency of the ion.

The Donnan laws are applicable to a system in a state of equilibrium. They do not describe the behaviour of a system not in equilibrium, but it is certain that a system containing indiffusible ions tends to reach a state described by the Donnan laws. Since the Donnan laws predict an uneven distribution of all types of diffusible ions, the presence or the formation of indiffusible ions in an initially homogeneous system must inevitably lead to a redistribution of the diffusible ions and to a concentration of either the negative or the positive ions, dependent on the sign of charge of the indiffusible ions. The relation to some of the accumulation phenomena in growing organisms is obvious.

Loeb's⁸⁾ important investigations on systems containing proteins showed that many of the properties of these systems were in agreement with the laws of the Donnan equilibrium. These investigations did not fail to attract the attention of physiologists. For a time several plant physiologists believed that the mineral nutrition of plants might be explained mainly on the basis of the Donnan theory. In Kostytschew's⁷⁾ textbook, for instance, the Donnan theory occupies an important place. However, many of these early expectations have not been fulfilled. As a result the Donnan principle has to some extent faded to the background. The purpose of this paper is to show that a denial of the applicability of the Donnan principle to plant physiology, simply because it cannot explain all the aspects of mineral uptake by plants, would be unjustified.

SOME SHORTCOMINGS OF THE DONNAN THEORY IN ITS SIMPLEST FORM

Of the substances contained in a plant root or other plant organ and held there by forces which need not further concern us, some are known to carry positively or negatively ionized groups. If such a plant organ is brought into contact with a medium containing ions, the essential conditions for the occurrence of some sort of Donnan system would seem to be fulfilled. The question may be asked whether the ionic relations between such a plant organ and its

medium are in agreement with the laws of the Donnan equilibrium. It appears that at least three major differences exist.

In the first place, the accumulation of ions in the i-phase of a simple Donnan system can only take place for ions of similar electrical charge. If, for example, indiffusible anions Z^- are present in a concentration of 0,08 n. and there is a diffusible electrolyte BA in the o-phase with a concentration of 0,03 n., the concentrations of B^- and A^- in the i-phase will be 0,09 and 0,01 n. respectively, because $0,03 \times 0,03 = 0,09 \times 0,01$. Thus the concentration of diffusible anions in the i-phase will be less than in the o-phase. In general such a situation is not observed in living organisms, where usually at least some of both negative and positive ions are found accumulated side by side. It might appear that Briggs' ¹⁾ conception of an i-phase consisting of more than one phase would be helpful here. He suggested that in a mature plant cell the vacuole is one phase and contains immobile cations, which do not diffuse because the cytoplasm is relatively impermeable to cations. As a result anions are accumulated in the vacuole. The cytoplasm itself is the other phase, which, due to its content of indiffusible anions, accumulates cations. The overall result would be that the concentrations of both the anions and the cations would, in the cell as a whole, be higher than in the medium. Though this picture certainly has its value for explaining the behaviour of mature cells with respect to changing composition of the surrounding mineral solution, it does not account for the accumulation of cations in the vacuole at an earlier stage. Neither does it provide a mechanism for the simultaneous accumulation of anions and cations in the xylem vessels of plant roots.

Secondly the laws of the Donnan equilibrium demand that ions of the same electrical charge and of equal valency shall have equal concentration ratios in the two phases and also that the product of the concentrations of pairs of ions having opposite electrical charge and equal valency shall be the same in both phases.

Thus $\frac{[Na^+]_i}{[Na^+]_o} = \frac{[K^+]_i}{[K^+]_o}$ (or, which is the same: $\frac{[Na^+]_i}{[K^+]_i} = \frac{[Na^+]_o}{[K^+]_o}$)
and $[K^+]_i \times [Cl^-]_i = [K^+]_o \times [Cl^-]_o$.

Both conditions are not satisfied in plants. The ratio of the potassium and sodium concentrations in a living organism may be widely different from their ratio in the medium. The process of

salt accumulation, i.e. the accumulation of both the anions and cations of a salt, indicates that equality of the products of ionic concentrations in the two phases is not realized either.

In the third place there is the fact that living organisms are in a continuous state of non-equilibrium; this could hardly be consistent with the existence of a true Donnan equilibrium.

A fourth point against the applicability of the Donnan theory has been raised by O s t e r h o u t ¹³). He argues that the osmotic pressure in the i-phase of any Donnan system would cause water to enter and dilute the contents, so that ultimately accumulation would be impossible. This objection is unjustified because it completely overlooks the existence of cohesional forces in the body substance of living organisms counteracting the effect of the osmotic suction.

EXPERIMENTAL EVIDENCE IN FAVOUR OF THE VALIDITY OF THE DONNAN PRINCIPLE FOR LIVING ORGANISMS

Before examining the evidence for the significance of the Donnan principle in biological systems it should be stressed that there is not the slightest reason to suppose that the partition of ions between a living organ and its medium has anything to do with an equilibrium state. It will be shown later that the Donnan equilibrium is used only because its laws are convenient to describe the relation between the ionic concentrations in the medium and in the outermost layer of a living organ, i.e. the layer which is in direct contact with the medium. The deeper layers of a tissue will have an ionic composition differing more from the composition of the surface layer the greater their distance from the outer surface of the tissue. It follows that the validity of the Donnan laws in relation to the distribution of ions between the two phases (tissue and external medium) cannot be tested by analyses of samples which include all parts of the body of the organism under consideration. Reversely, such analyses would not demonstrate that the Donnan equilibrium cannot be used to describe ionic relationships between the surface layer of the tissue and the external medium.

It thus appears that it will not be possible, by the usual methods of analysis, to demonstrate whether the distribution of ions on each side of the boundary between a living tissue and the external medium is in accordance with the Donnan equilibrium. Fortunately

another property of Donnan systems, the membrane potential, seems to offer better means of approach. The membrane potential is situated at the boundary between the two phases of a Donnan system and its magnitude is directly related to the ratio of the ion concentrations on either side of the boundary. The measurement and interpretation of these membrane potentials in plants does not seem to have received sufficient attention. Whilst it is true that a number of surface potentials of living plants have been measured, some of them undoubtedly being membrane potentials or partly so, they have seldom been properly identified. Hellinga⁵⁾ summarized a number of investigations on the electrical charges of plants or plant organs, many of them showing very conflicting results. Lundegårdh⁹⁾, (p. 258–263) enumerated several reasons for this confusion: sometimes the electrical charge of the medium has been mistaken for the charge of the organ, in other cases electrodes with ill-defined or irreproducible contact potentials have been used or surface potentials have been incorrectly interpreted as diffusion potentials or electrostatic phenomena. It may also be added that sometimes an asymmetrical set of electrodes has been employed.

Lundegårdh's interpretation of the potentials he measured on roots of cereals has considerably clarified the nature of these potentials. The method of measurement employed warrants the conclusion that in the potential differences observed only the properties of the surface of the root are involved. In his latest paper on the subject Lundegårdh¹⁰⁾ assumed the root potential to be essentially a Donnan membrane potential. This idea was taken up by Tendeloo, Vervelde & Zwart Voorspuy^{14) 15)}, who, with some adjustments, arrived at an interpretation not very different from Lundegårdh's. The potentials measured were compared with theoretical potentials which can be calculated for a Donnan system, whose i-phase contains a certain concentration of a weak acidoid. The non-diffusible ions originate from the dissociated fraction of the acidoid. As the dissociation depends on the pH, the concentration A varies with changes in hydrogen ion concentration. Vervelde¹⁶⁾ adapted the theory to the more general case of an i-phase containing a mixture of weak acidoids. A linear relationship between the concentration of the non-diffusible ions and the pH, of the form

$$A = K_1 \log c_H + K_2 \quad (1),$$

gave a good agreement between the theoretically calculated potentials and the potentials actually measured in KCl-solutions of different concentrations. Table I gives some of the values obtained.

TABLE I

Potential measurements on roots in solutions of neutral KCl							
Plant	conc. KCl →	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
Rye (<i>Secale cereale</i>) variety "Petkuser Kortstro"	$E_{\text{exp.}}$		207,5	186,8	149,8	126,3	123,5
	$E_{\text{calc.}} \begin{cases} K_1 = -0,0114 \\ K_2 = -0,0856 \end{cases}$	218,0	212,8	186,8	149,9	126,2	119,1
Wheat (<i>Triticum vulgare</i>) variety "Juliana"	$E_{\text{exp.}}$		197,3	183,0	155,5	126,3	118,0
	$E_{\text{calc.}} \begin{cases} K_1 = -0,0089 \\ K_2 = -0,0710 \end{cases}$	207,5	203,7	182,9	148,1	126,3	120,2
Oats (<i>Avena sativa</i>), variety "Adelaar"	$E_{\text{exp.}}$		189,3	175,8	143,7	111,0	104,6
	$E_{\text{calc.}} \begin{cases} K_1 = -0,0057 \\ K_2 = -0,0439 \end{cases}$	196,0	193,5	175,9	140,6	113,5	104,1
Barley (<i>Hordeum vulgare</i>) variety "Groninger wintergerst"	$E_{\text{exp.}}$		186,8	175,8	152,0	124,3	118,7
	$E_{\text{calc.}} \begin{cases} K_1 = -0,0053 \\ K_2 = -0,0459 \end{cases}$	190,5	188,6	176,1	145,9	125,4	120,0

Each experimental value in the table is the average of six values measured on different roots. The theoretical values for the potentials have been calculated by assuming that the system being investigated can be regarded as a Donnan equilibrium, whose i-phase is characterized by equation (1) with the values of K_1 and K_2 appearing in the table.

This picture was further extended by Vervelde & Tendela¹⁷⁾ to account for the reversal of the sign of the root potentials at low pH values. Some of the pertinent figures are given in table II.

TABLE II

Root potentials in mV, measured in solutions of HCl.						
Plant species	Number of roots	pH and corresponding potential				
Rye (<i>Secale cereale</i>)	10	pH . . . 5,27	4,15	3,14	2,11	1,15
		pot . . . -87	-43	-5	+17	+38
Rye (<i>Secale cereale</i>)	5	pH . . . 5,08	4,12	3,09	2,07	1,10
		pot . . . -58	-28	+3½	+19	+22
Wheat (<i>Triticum aestivum</i>)	3	pH . . . 5,08	4,12	3,09	2,07	1,10
		pot . . . -58	-28	+8	+29	+45

The reversal of the sign of the potential was shown to be the result of the amphoteric nature of the root substance. At low pH values

there are no indiffusible anions, but indiffusible cations. The Donnan laws then predict a reversal of the sign of the membrane potential under these conditions as compared with the potential under less acid conditions. It was shown that the equation (1) can still be used, because the quantity A in this equation becomes negative at low pH values and a negative concentration of indiffusible anions is equivalent to a positive concentration of indiffusible cations.

These potential measurements have been carried out on living roots and there will no doubt have been transport of ions through the membrane during the measurements. In spite of this, however, the laws of the Donnan equilibrium are still applicable and the following physico-chemical considerations indicate that this is nothing other than could be expected.

Migration of diffusible ions in a system occurs if the electrochemical potential, as defined by Guggenheim⁴⁾, for these particular ions shows differences from place to place. Expressed more exactly: at a given place the rate of transport of a diffusible ion in a certain direction is proportional to the gradient of its electrochemical potential in that direction. Consequently there can be no large variation in the level of the electrochemical potential from place to place, since such differences would mean an extremely steep potential gradient which would result in a very explosive movement of ions over the gradient thereby destroying the gradient. For the same reason no fall in the electrochemical potential can be expected to occur at a root wall^{*)}. If the electrochemical potential gradient for a given kind of migrating ion were measured at right angles to the root surface, it would therefore not be of the form represented by curve (a) in fig. 1, but it would have a form similar to that in curve (b). The potential gradient may differ on either side of the surface, due to differences in transport capacities, yet the curve as a whole will be continuous. If the boundary be regarded as a mathematical plane the electrochemical potentials on either side will be exactly equal. Even if there is a root wall with a certain small thickness both potentials will be very nearly equal. *The equality of electrochemical potentials on either side of a membrane separating two phases having different concentrations of indiffusible*

*) The theoretical approach to the ionic relationships between plant roots and an external aqueous medium as given here may also be applied to single cells in contact with a salt solution.

ions (ions unable to permeate through the membrane) is the very basis of the laws of the Donnan equilibrium. Thus, for a situation where transport of ions occurs across such a membrane, the Donnan laws are fully valid, provided they are only applied to infinitesimal layers on either side. As in the case of a Donnan system which has reached a true equilibrium state, there is an electrical potential difference between the two phases and consequently, in view of the equality of the electrochemical potentials, the chemical potentials in the two phases are different, that is the ion concentrations are unequal.

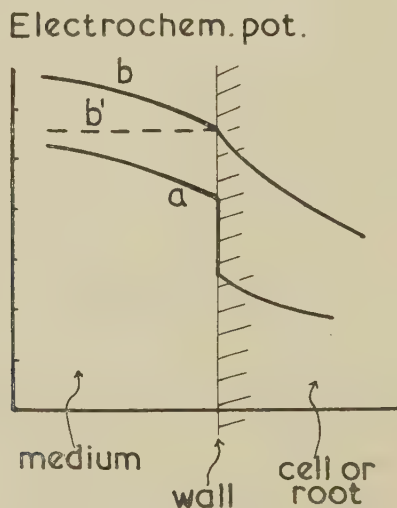


Fig. 1. Diagrammatic representations of the possible variations in electrochemical potential from the external medium (o-phase) to the interior of the root (i-phase) through the outer wall of a surface cell. (a) variations of this type are considered highly improbable, (b) the normal type of variation, (b') the variation when the external medium (o-phase) is stirred.

The restriction of the applicability of the Donnan laws to infinitesimal layers on either side of the boundary between the phases is not necessary for the phase outside a living organism if care is taken to prevent the occurrence of gradients in the electrochemical potential by adequate stirring (curve *b'* in fig. 1). Under these conditions the relation between the composition of the surface layer of the root and the composition of the nutrient solution as a whole can be fully described by the Donnan laws.

This implies that the first step of ion intake by living organisms can be quantitatively understood.

Another argument in favour of the applicability of the Donnan theory to processes of living organisms has been advanced by Elgabaly & Wiklander³⁾ and will be discussed in the next section. Additional evidence may be obtained by a method recently described by Hope & Stevens⁶⁾, whereby the KCl concentration in root tips is measured as a function of the external KCl concentration and of the pH.

SOME CONSEQUENCES OF THE APPLICABILITY OF THE DONNAN PRINCIPLE

It will be seen that, according to the theory set out above, the ionic composition of the outermost layer of the root is such, that

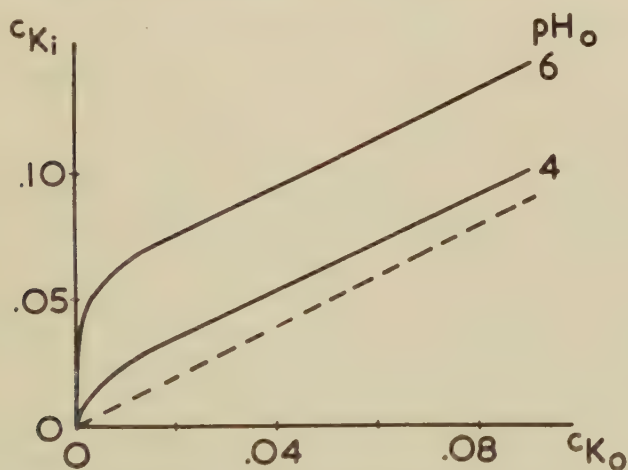


Fig. 2. Curves showing the theoretical relationship between accumulation of potassium in the i-phase of a Donnan system and the hydrogen ion and potassium ion concentrations in the external medium (o-phase).

--- curve of the relationship when there is an even distribution of ions between the two phases.

ions of one sign of charge are accumulated in the surface layer of a tissue, just as they are accumulated in the i-phase of a true Donnan equilibrium. There is no reason why this type of accumulation should not be regarded as adsorption. Thus the picture given is in perfect accordance with the common opinion that the first step of

the uptake of ions by living organs is an adsorption. It should be noted, however, that the type of adsorption described here has a strictly specified nature and mechanism, because it is attributable to the presence of indiffusible ions of opposite charge and not to some unspecified adsorption force.

Otherwise, the quantitative aspects of the Donnan-type of adsorption are rather similar to those found for adsorption phenomena in general. To illustrate this, some calculations have been made for one particular hypothetical Donnan system, having the following values for the two constants K_1 and K_2 of equation (1): $K_1 = -0,03$ and $K_2 = -0,09$. If the o-phase (the medium surrounding the root) be supposed to contain KCl and some HCl to regulate the pH in the o-phase (pH_0), the relation between the potassium concentrations in both phases for a few pH_0 values is expressed by the curves of fig. 2. The broken line has been drawn in for comparison; it expresses an even distribution of ions (no adsorption). Obviously, the adsorption ratio $\frac{[\text{K}^+]_i}{[\text{K}^+]_0}$ is highest at

low potassium concentrations in the medium, just as it has been found for nearly all adsorption phenomena. The adsorption ratio increases with a rise in pH_0 . This influence of pH_0 on the adsorption is demonstrated more clearly in fig. 3, showing that at higher pH_0 -values the adsorbent is more saturated with potassium.

As has been indicated in the preceding section, it should not be supposed that the ionic composition of an entire organism or organ obeys the laws of the Donnan equilibrium. Yet, since the organism undoubtedly tends to reach a state of ionic equilibrium with the medium (which equilibrium has already been reached in the surface layer), it will not be surprising if a rough reflection of some of the major Donnan effects is found when the ionic composition of entire organs is compared with the composition of the external medium. An interesting example of what may be such a reflection has been described by Elgabaly & Wiklander³⁾. They found a preferential uptake of divalent calcium ions by pea roots as compared to a preference of barley roots for the monovalent sodium ion. This difference was attributed to a greater content of acidoids (more or less comparable to the negative indiffusible ions in our theory) in pea roots. The preferential accumulation of polyvalent ions in phases with a high concentration

of indiffusible ions is a well known Donnan effect. The relatively high content of bases, particularly divalent bases, in leguminous plants is a well established fact. The indication that the content of indiffusible ions is related to the protein content is certainly worth following up.

The picture given here of a plant organ as the i-phase of a Donnan system implies that the presence of the indiffusible ions is not restricted to surface layers of the tissue or the organ. There is indeed no reason to suppose that the adsorption processes of the interior tissues of roots (processes which are commonly called absorption

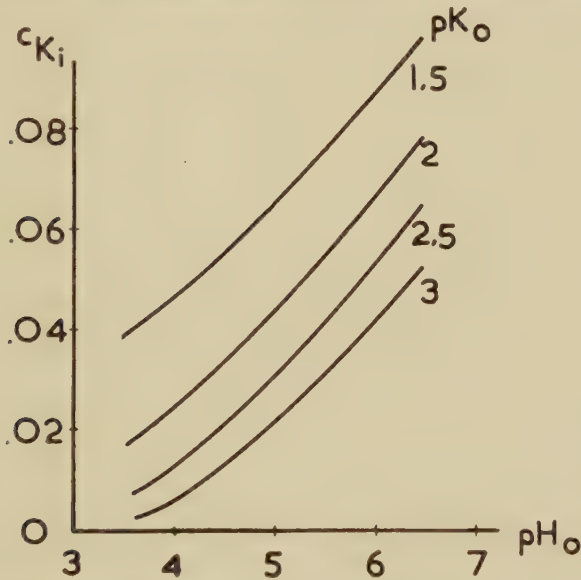


Fig. 3. Curves showing the relationship between the accumulation of potassium in the i-phase and the pH of the external medium (o-phase) for a number of different potassium ion concentrations in the external medium (o-phase).

processes because of their spatial nature) are fundamentally different from those of the surface layer, though, of course, there may be quantitative differences. Thus, any theory on the migration of ions in living organisms should also make allowance for the presence of the same forces in the deeper layers of the organism as the adsorption forces on the surface.

The Donnan theory also enables something to be said about the

"anion respiration" theory for the uptake of ions by roots. This theory was originally based on the observation by Lundegårdh & Burström^{11) 12)} that there is a very close quantitative relationship between the respiration rate (rate of CO₂ production) of roots and the rate of anion uptake, whereas no such close relation was found between respiration and cation uptake. From the effects of respiratory poisons, which inhibited both the anion uptake and part of the aerobic respiration, Lundegårdh adduced further evidence in support of his view that the anion uptake is intimately linked with the poison sensitive part of the respiration, which was therefore called anion respiration. In later publications a mechanism to explain the relationship between anion respiration and anion uptake was suggested. The transport of anions was held to be related to the change of valency of iron atoms in a hemin respiratory system; each iron atom changing its valency being responsible for the displacement of one anion. As the respiratory enzyme acts upon the substrate, energy is obtained to raise the osmotic level of the anions. If this theory is correct, the question arises as to why energy is needed for the accumulation of anions and not for the accumulation of cations. Lundegårdh^{9) 10)} demonstrated beyond any doubt that plant roots, under normal conditions, are negatively charged with respect to the external medium. This charge is, in fact, the electrical potential difference between the two phases of the Donnan system as described above. Lundegårdh⁹⁾ (p. 301) considers that the negative potential favours the entrance of positively charged cations into the root with the result that the uptake of cations by plants would be a passive process requiring no special source of energy. On the other hand the same electrical potential would be an obstacle to the entrance of anions into the root, so that movement of anions from the medium into the tissue would require energy to overcome the electrical repulsion. This energy would be supplied by the anion respiration.

It would seem that this picture is incorrect. It is true that the entrance of anions is impeded by the electrical potential fall at the surface, but there is at the same time a concentration fall favouring the entrance of anions. Reflection will show that the repellant action of the electrical charge is exactly balanced by the accelerating effect of the concentration fall, because this is implied in the above

mentioned equality of the electrochemical potential on either side of the root wall. The energy required to overcome the electrical potential difference is, at the same time, made available by the transfer of anions to a lower osmotic energy level. Obviously, the mere passage of anions through the walls of the outer cells of the root does not require the intervention of a special "anion respiration" to supply energy. This does not preclude the possibility that "anion respiration" is active in another phase of the process of anion uptake.

Similar reasoning is valid for the uptake of cations. Their inward migration is favoured by the electrical potential difference, but retarded by the concentration difference. Though the passage of cations through the walls of the outer cells of a root is not an energy-consuming process, neither can it be regarded as a passive energy-releasing process.

SUMMARY

Serious objections have been raised by different investigators against the assumption that a living organism can be regarded as in a state of Donnan equilibrium with the external aqueous medium. Yet, observations on the surface potentials of plant roots show that the ionic distribution across the exterior walls of the outer cells of the root is in accordance with the laws of the Donnan equilibrium. It is pointed out that this type of ionic distribution is valid for infinitesimal layers on either side of the root surface, because there exists, across the surface cell wall, an electrochemical potential gradient without any sudden differences. This means that the electrochemical potential is about the same for the outer layer of living organisms in contact with a nutrient solution as for the solution itself. This equality of electrochemical potentials, in combination with the occurrence of indiffusible ions inside the wall, fulfills the conditions for the establishment of a Donnan distribution. As the Donnan distribution may be regarded as adsorption (or, if one likes, absorption), this picture confirms the idea that the first step of ion uptake is an adsorption on to cell constituents. The adsorptive forces are not restricted to surface layers. Therefore, though entire organs do not reach a state of equilibrium with the medium, the tendency to approach such an equilibrium is indicated from the occurrence of ionic compositions of plant roots or plant organs which are in agreement with the requirements of the Donnan theory. An example of this is to be seen in the preferential uptake of divalent ions by plant roots with high acidoid contents.

From the applicability of the Donnan laws to the ionic distribution between the root and the external medium it can be deduced that no

special energy is required to carry an anion through the wall against the negative charge of the root surface. The energy needed to overcome the negative potential is derived from an equivalent decrease in the osmotic energy level. The negative charge of root surfaces cannot be used as an argument in favour of Lundegårdh's anion respiration theory.

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GRUNDLEGENDE BEMERKUNGEN ZUM „UM-
KEHRBAREN ERTRAGS-STICKSTOFF-GESETZ“
VON O. W. WILLCOX. (THE INVERSE YIELD-
NITROGEN LAW)

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Es ist das grosse Verdienst von O. W. Wilcox, dass er die Bedeutung des Wirkungsgesetzes der Wachstumsfaktoren, welches vor nunmehr über 40 Jahren von Mitscherlich aufgestellt wurde, den amerikanischen Kollegen nahe gebracht hat. Ist doch durch dieses Gesetz die Möglichkeit gegeben, die Steigerung der Pflanzenerträge quantitativ auszuwerten, und damit von der Ertragssteigerung eines Jahres, die durch eine bestimmte Düngerart und Düngermenge erzielt wird, auf die Ertragssteigerungen der folgenden Jahre zu schliessen. Das Wirkungsgesetz zeigt, wie der Pflanzenertrag nicht nur von einem, dem Minimumfaktor Liebig's, sondern gleichzeitig von allen Wachstumsfaktoren in seiner Höhe bedingt wird. Für die Übertragung der Ergebnisse von einem Jahr auf das andere, so von einem Felddüngungsversuch, oder von einem Gefässdüngungsversuch auf die Ertragssteigerung, die wir im nächsten Jahr durch eine Düngung im Felde erzielen müssen, ist nun die Konstanz des Wirkungsfaktors Vorbedingung. Diese Konstanz des Wirkungsfaktors lässt sich pflanzenphysiologisch nur a posteriori, nicht a priori feststellen. Sie ist, auf Grund von vielen tausenden Versuchen, so lange sichergestellt, wie nicht, durch die Einwirkung irgend eines anderen Faktors, bei der Ertragssteigerung eine Ertragsdepression statt hat. In der landwirtschaftlichen Praxis tritt nun eine derartige Ertragsdepression bei Düngungsversuchen mit Phosphorsäure und Kali auf den verschiedensten Bodenarten kaum ein, während sie bei der Stickstoff-

düngung, durch zu starke Gaben zu Beginn der Vegetation, leicht auftreten kann. So lässt sich die Bestimmung des Wirkungswertes für Kali mit $c = 0,4$ je dz/ha, und für Phosphorsäure mit $c = 0,6$ je dz/ha, mit grosser Sicherheit ermitteln, während der Wirkungswert für den Stickstoff nicht so sicher festzustellen war. Aus diesem Grunde haben Mitscherlich und Atanasiu¹⁾ nochmals das ganze vorliegende Material über den Wirkungswert des Stickstoffs von neuem verarbeitet, wobei sie feststellen konnten, dass eine Erhöhung des Wirkungswertes von $c = 0,122$ je dz/ha auf $c = 0,2$ je dz/ha richtiger erschien. O. W. Willcox²⁾ hat gegen diese Erhöhung des Wirkungswertes Einspruch erhoben, da diese nicht mit seinem „Umkehrbaren Ertrags-Stickstoff-Gesetz“ in Einklang zu bringen ist. Eine eingehende Verarbeitung der Willcox'schen Arbeiten zeigt nun grosse Differenzen in unseren wissenschaftlichen Auffassungen, die geklärt werden müssen, was nachfolgend versucht wird:

Willcox³⁾ schreibt:

„Das Mitscherlich'sche Ertragsgesetz — für sich allein betrachtet — bedeutet keine vollständige Erklärung der Wachstumskräfte und des Ertrages, weil dieses Gesetz, für sich selbst, keine Formulierung zur Ermittlung der Höchstgrenze des quantitativen Lebens (Q) — das von jeder Pflanze ganz spezifisch hervorgerufen wird — schafft. Mitscherlich's Ertragsgesetz ist stumm, jedenfalls was die oberste Grenze des A-Wertes anbetrifft. Von hier ab stehen wir unter der Autorität des umkehrbaren Ertrags-Stickstoff-Gesetzes, das — ähnlich dem Gesetz vom abnehmenden Ertragszuwachs — auf Wurzeln und Blätter der Pflanze Anwendung findet. Das Umkehrbare Ertrags-Stickstoff-Gesetz besagt folgendes:

„Wenn zwei oder mehr Pflanzenarten auf demselben normalen Boden gewachsen sind — der frei von schädlichen Einflüssen ist — muss die Pflanze mit dem geringsten prozentischen Stickstoffgehalt in der gesamten oberirdischen Trockenmasse den höchsten Ertrag erbringen. Oder anders ausgedrückt: Die Pflanzenerträge stehen im umgekehrten Verhältnis zu ihrem prozentischen Stickstoffgehalt, was soviel heisst, dass je geringer der prozentische Stickstoffgehalt ist, umso höher der Ertrag ausfallen wird“.

Durch das „Umkehrbare Ertrags-Stickstoff-Gesetz“ sucht O. W. Willcox den absoluten Höchstertrag, den man durch eine

Stickstoffdüngung zu erzielen vermag, zu bestimmen. Nach dem Mitscherlich'schen Wirkungsgesetz ist etwas derartiges nicht möglich, denn der absolute Höchstertrag A_n (nach Willcox Q_n) wird nach der folgenden Gleichung (1), die auch Willcox anerkennen, erhalten:

$$y = A_n(1 - 10^{-c_1 \cdot x_1})(1 - 10^{-c_2 \cdot x_2})(1 - 10^{-c_3 \cdot x_3}) \dots (1 - 10^{-c_n \cdot x_n}) \quad (1)$$

Es ist nun aber nicht statthaft, die weiteren Glieder der Gleichung (1) wegzulassen, und nur das erste Glied, welches nur den Wirkungsfaktor c_1 und die Stickstoffmengen x_1 berücksichtigt, stehen zu lassen, wobei sich fälschlicherweise die folgende Gleichung (2) ergibt:

$$y = Q_n(1 - 10^{-c_1 \cdot x_1}) \quad (2)$$

Nach dem Wirkungsgesetze wird der absolute Höchstertrag A_n (bzw. Q_n) nie festzustellen sein. Er hängt also keineswegs ausschliesslich von den aufnehmbaren Stickstoffmengen ab! Er wird wesentlich höher, wenn sich alle anderen Wachstumsfaktoren, so nicht nur die verschiedenen Nährstoffe, sondern auch die Witterungsverhältnisse, das Wasser, die Wärme, das Licht und die physikalischen Bodenverhältnisse günstiger gestalten, wenn die Pflanzenart und die Pflanzenzüchtung ertragreicher sind, u.a.m.

Bekanntlich lautet nun die integrierte Form des Wirkungsgesetzes:

$$\log(100 - y) = \log 100 - c \cdot x \quad (3)$$

TABELLE I

Prozentische Ertragstabelle für Stickstoff		
y in Prozenten des Höchstertrages	x in dz/ha, wenn	
	c = 0,122	c = 0,2 ist *)
50	2,5	1,5
75	5,0	3,0
87,5	7,5	4,5
93,75	10,0	6,0
96,875	12,5	7,5
98,437	15,0	9,0
99,218	17,5	10,5
99,609	20,0	12,0
99,80	22,5	13,5
99,99	25,0	15,0

*) Die N-Mengen sind hier einmal für den Wirkungswert $c = 0,122$, sowie für unseren neuen Wirkungswert $c = 0,2$ angegeben.

Mit Hilfe dieser Gleichung kann man eine Ertragstabelle für Stickstoff aufstellen, und dann, wie das Willcox getan hat, mit den sogenannten Baule-Einheiten rechnen, wobei eine Baule-Einheit die Nährstoffmenge ist, mit der gerade die Hälfte (50%) des Höchstertrages erreicht wird.

Die sich daraus ergebenden Folgerungen sind allgemein bekannt. Mit den ersten Nährstoffeinheiten (N.E.) erreicht man 50% des Höchstertrages, mit zwei N.E. $50\% + 25\% = 75\%$, mit drei N.E. $75\% + 12,5\% = 87,5\%$ u.s.f. Oder anders ausgedrückt: Der Ertragszuwachs wird nach der logarithmischen Funktion immer kleiner. Man erkennt daraus, dass zu zehn Nährstoffeinheiten $= 25 \text{ dz/ha}$ bei $c = 0,122$, oder 15 dz/ha bei $c = 0,2$, erforderlich sind, um 99,99'', bzw. den Q -Wert nach Willcox, also den A -Wert = den Höchstertrag, zu erreichen.

Von hier ab geht Willcox³⁾ wieder neue Wege, die u.E. mit den Grundlagen der Pflanzenphysiologie nicht in Einklang zu bringen sind. Er kombiniert nämlich mit dem abnehmenden Ertragszuwachs die Stickstoffaufnahme der Pflanzenerträge, und kommt dabei zu folgender Schlussfolgerung:

„Daher ist bei der kombinierten Wirkung des Ertragsgesetzes und „des „Umkehrbaren Ertrags-Stickstoff-Gesetzes“ eine Baule-Einheit „an Stickstoff ausreichend, um die Hälfte (50%) des maximal möglichen Ertrages an Protein bei jeder Pflanze in einem Wachstumsrhythmus zu erzielen“.

Es verwundert daher weiter nicht, wenn er annimmt:

„Es besteht ein fester Zusammenhang zwischen den Mengen eines „Nährstoffes (N) im Boden, und der Menge an Protein, die pro „Einheit eines normalen Bodens produziert wird, ohne Rücksicht „auf die Pflanzensorte, und ohne Rücksicht auf die Mengen an „N-haltigen Material, das die Pflanzen produzieren“:-

Für diese Behauptung führt Willcox zwei Beweise an, die im einzelnen untersucht und besprochen werden sollen:

Der erste Beweis wird rein spekulativ aus der prozentischen Ertragstabelle für Stickstoff (Seite 325) abgeleitet. Willcox nimmt nämlich an, dass von den Nährstoffeinheiten in ähnlicher Weise nur eine ganz bestimmte N-menge aufgenommen wird, etwa in folgender Weise:

TABELLE II

		wenn $c = 0,122$		wenn $c = 0,2$
		nach Willcox	richtiger	
Stickstoff von d.1. Gabe etwa	2,4 dz/ha	2,4 dz/ha	1,50 dz/ha	
Stickstoff von d.2. Gabe etwa	0,62 dz/ha	1,2 dz/ha	0,75 dz/ha	
Stickstoff von d.3. Gabe etwa	0,31 dz/ha	0,6 dz/ha	0,39 dz/ha	
Stickstoff von d.4. Gabe etwa	0,15 dz/ha	0,3 dz/ha	0,19 dz/ha	
Stickstoff von d.5. Gabe etwa	0,07 dz/ha	0,15 dz/ha	0,10 dz/ha usw.	
		$\approx 3,60$ dz/ha	$\approx 4,80$ dz/ha	$\approx 3,00$ dz/ha

Man erkennt aus dieser Tabelle II unschwer, dass die aufgenommenen N-Mengen immer kleiner werden. Warum aber Willcox nach der ersten bei der zweiten N-Gabe nur 1/4 der Ersteren — statt der Hälfte — zum Ansatz bringt, bleibt offen und ungeklärt. Die Menge von 3,60 dz/ha ist also rein willkürlich gewählt. Zu dem Werte 3,60 kann so Willcox nur kommen, wenn er bei der ersten Gabe mit 1,80 beginnt — Zum Vergleiche haben wir auch die Werte für den Wirkungswert $c = 0,2$ mit angegeben, wobei wir zu Werten kommen, die dem Anfangswerte 1,80 und der Endsumme 3,60 wesentlich näher liegen würden, als 2,4 bzw. 4,8 dz/ha! —, sodass darum sein „Umkehrbares Ertrags-Stickstoff-Gesetz“ noch nicht zu fallen braucht!

Den zweiten Beweis leitet Willcox aus der Ertragsgleichung (3) ab. Nach x hin aufgelöst, lautet sie:

$$x_N = \frac{\log 100 - \log (100 - y)}{c} \quad (4)$$

Hiernach ist x eine Funktion von y , wobei x_N die N-Mengen angeben soll, die in Wirklichkeit von den Pflanzen aufgenommen und bei der Ernte dem Felde entzogen werden. Für c benutzt Willcox den alten Wirkungswert $c = 0,122$. Wenn Willcox nun diese Gleichung (4) nach x hin auflöst — für den Fall, dass $y = 100$ ist — so erhält er einen x_N -Wert von 3,56 dz/ha, der — so folgert er — mit der oben angegebenen Annäherungszahl 3,60 so leidlich übereinstimmt. Diesen Überlegungen können die Verfasser nicht folgen, weil nämlich für den Fall, dass $y = 100$ ist, $x = +\infty$ wird, und zwar ganz unabhängig davon, wie hoch der Wirkungswert „ c “ ist! Wenn y dagegen nicht 100, sondern 63,2 ist, erhält man für $x = 3,56$ dz/ha, denn es ist

$$3,56 = \frac{\log 100 - \log (100 - 63,2)}{0,122}$$

Wählt man für $y = 99,99$, so muss der x -Wert (für 10 Nährstoffeinheiten), wie wir zuvor zeigten, 25 dz/ha sein.

In beiden Fällen ist somit die sogenannte Agrobiologische Stickstoffkonstante x_N mit 3,60, bzw. mit 3,56, völlig willkürlich angenommen worden. Der in diesen beiden Fällen bei Willcox übereinstimmende Wert, ist weder nach der einen, noch nach der anderen Methode haltbar, oder zu begründen.

Diese kurzen Darlegungen der Willcox'schen Theorien waren notwendig, um seine nun folgenden Schlussfolgerungen zu verstehen. Er schreibt darüber:

Diese Grenzmengen an aufnehmbarem Bodenstickstoff wurden mit 3,56 dz/ha berechnet ⁴⁾. Unter Annahme des Agrobiologischen Stickstofffaktors 356, können nun die Lebensverhältnisse einer jeden Pflanze durch die Formel $356/N$ gefunden werden, wobei N der normale prozentische Stickstoffgehalt ist, den die pflanzliche Substanz enthält ⁵⁾.

Wenn man also die Ertragsfähigkeit irgendeiner Pflanze bestimmen will, braucht man nach Willcox weiter nichts zu tun, als den prozentischen N -Gehalt zu bestimmen, und durch diesen dann die Agrobiologische Stickstoffkonstante 356 zu dividieren. Nach Abzug der Blätter, Stengelteile usw. der betreffenden Pflanze, kann man dann sofort sagen, wie hoch der maximale Höchstertrag ausfallen muss.

Wie aus den bisherigen Ausführungen klar hervorgeht, spielt der alte Wirkungswert $c = 0,122$ bei der Berechnung der Willcox'schen Agrobiologischen Stickstoffkonstanten (q) eine grosse Rolle. Denn mit einer Änderung dieses c -Wertes — ob negativ oder positiv — werden auch seine Höchsterträge beeinflusst. Setzt man den c -Wert niedriger an, so erreichen die A -Werte fantastische Höhen; umgekehrt müssen die A -Werte bei Vergrösserung des c -Wertes niedriger ausfallen, und im extremen Falle so niedrig sein, dass die tatsächlichen Erträge bereits über den A -Werten liegen.

So ist es also durchaus verständlich, dass sich Willcox mit aller Schärfe gegen die Heraufsetzung des c -Wertes auf 0,2 wendet, denn dadurch muss automatisch das eintreten, was soeben ausgeführt wurde.

bei $c = 0,122$ ist $Q = 356/N$

bei $c = 0,2$ ist $Q = 217/N$

Zwei Beispiele von Willcox mögen das verdeutlichen:

1.) Eine Weizensorte mit 1,25% N, müsste danach folgende Höchsterträge bringen:				
	$c = 0,122$		$c = 0,2$	
bei einem Korn: Strohverhältnis von				
40 : 60, ...	285 dz/ha		178	dz/ha
wären das an reinem Weizen:	114 dz/ha	bzw.	69,4	dz/ha
2.) Hat eine Zuckerrübe (Wurzel, Blätter und Kopf) einen prozentischen N-Gehalt				
von 0,21%, so liegt die maximale Ertrags-			bzw.	
fähigkeit bei:	356/0,21 = 1695 dz/ha		217/0,21 = 1004 dz/ha	
bringt man $\frac{1}{2}$ davon für Blätter in Abzug,				
ergeben sich ha-Erträge von:	1130 dz/ha	bzw.	690 dz/ha	

In ebenderselben Weise kann man nach Willcox für jede Pflanzenart die maximale Ertragsleistung bestimmen.

Wenn man mit Fug und Recht annehmen darf, dass eine solche Koppelung des Wirkungsgesetzes der Wachstumsfaktoren mit einer Agrobiologischen Stickstoffkonstanten (Q) unvereinbar ist, so bleibt doch die Frage offen, ob die Willcox'sche Theorie zutrifft, wonach sich der prozentische Stickstoffgehalt in den Pflanzen umgekehrt proportional zum Gesamtertrage verhält.

Das dem nicht so ist, soll zum Schlusse gezeigt werden:

Aus einer Fülle von vorliegendem Untersuchungsmaterial sind zwei Feld-, und ferner ein Gefäß- und ein Nährlösungsversuch ausgewählt worden, mit deren Hilfe die Theorien von Willcox widerlegt werden sollen. Dabei wollen wir wieder von einer gemeinsamen Basis ausgehen, die auch Willcox akzeptiert, nämlich, dass die Ertragssteigerung nach logarithmischer Funktion verläuft.

Willcox nimmt nun an, dass — wenn die Stickstoffgabe gleich bleibt — dann der prozentische Stickstoffgehalt der Pflanzen mit der Steigerung der Erträge durch andere Wachstumsfaktoren fällt; dass er aber angenähert gleich bleibt, wenn die Erträge durch erhöhte Stickstoffgaben steigen. Wir haben, wie wir das nachfolgend beweisen werden, gerade das Gegenteil feststellen müssen, nämlich, dass mit der Steigerung des Ertrages durch eine N-Düngung, auch der prozentische N-Gehalt der Pflanze nach logarithmischer Funktion *ansteigt* wodurch ein Grenzwert im absoluten N-Gehalte der Pflanze kaum feststellbar ist.

Es ist somit auch der von Willcox festgestellte Grenzwert von 3,56 dz ha N = 2222 kg ha Rohprotein, keineswegs der wirkliche Höchstwert. Zum Beweise hierfür möge zunächst ein von Mitscherlich ausgeführter N-Düngungsversuch mit Runkelrüben dienen, dessen Ergebnisse folgen ⁵⁾:

TABELLE III

Wurzel-Erträge									
Dün- gung dz/ha N	Wurzel-Erträge dz/ha		Trockensubst. dz/ha		% N		dz/ha N		
	gefund.	berechn.	gefund.	berechn.	gefund.	berechn.	gefund.	berechn.	
1	2	3	4	5	6	7	8	9	
0,0	584	525	57,8	57,8	1,29	1,29	0,75	0,75	
0,4	596	607	66,8	66,7	1,42	1,42	0,95	0,96	
0,6	668	681	74,9	74,7	1,53	1,53	1,15	1,16	
1,2	723	747	82,2	82,0	1,64	1,63	1,35	1,35	
1,6	846	806	88,7	88,5	1,73	1,72	1,53	1,53	
2,0	853	858	94,4	94,4	1,81	1,80	1,71	1,70	
2,4	897	905	100,0	99,8	1,88	1,88	1,88	1,86	
3,0	970	967	106,4	106,9	1,98	1,97	2,11	2,09	
4,0	1054	1048	115,3	116,5	2,10	2,10	2,42	2,43	

Zur Fortsetzung:

Dün- gung dz/ha N	Blatterträge dz/ha		Trockensubst. dz/ha		% N inter- poliert	dz/ha N		N in der ges. Ernte dz/ha	
	gef.	ber.	gef.	ber.		gef.	ber.	gef.	ber.
1	10	11	12	13	14	15	16	17	18
0,0	113	65	7,2	7,2	1,93	0,14	0,14	0,89	0,89
0,4	102	98	10,8	10,7	2,08	0,22	0,24	1,18	1,18
0,8	120	128	14,1	13,8	2,22	0,31	0,33	1,45	1,45
1,2	151	154	16,9	16,7	2,34	0,40	0,41	1,72	1,72
1,6	190	177	19,5	19,3	2,46	0,48	0,49	1,97	1,98
2,0	190	198	21,8	21,7	2,55	0,56	0,57	2,22	2,23
2,4	212	217	23,9	23,8	2,64	0,63	0,61	2,45	2,45
3,0	249	242	26,6	26,7	2,76	0,73	0,73	2,79	2,79
4,0	274	274	30,1	30,6	2,91	0,87	0,87	3,30	3,30

Zunächst die einzelnen Gleichungen für die berechneten Werte:
(Nach Rubriknummern geordnet)

Zu Rubrik: (3) $\log (1300 - y) = 2,8893 - 0,122 \cdot x$

(5) $\log (150 - y) = 1,9647 - 0,11 \cdot x$

(7) $\log (2,50 - y) = 0,0828 - 0,12 \cdot x$

(9) $\log (4,70 - y) = 0,5966 - 0,06 \cdot x$

(11) $\log (375 - y) = 1,4914 - 0,122 \cdot x$

(13) $\log (45 - y) = 1,5775 - 0,105 \cdot x$

(14) $\log (3,40 - y) = 0,1673 - 0,12 \cdot x$

(16) $\log (1,40 - y) = 0,1644 - 0,075 \cdot x$

(18) $\log (8,00 - y) = 0,8519 - 0,045 \cdot x$

Obwohl in diesem Düngungsversuch N-Mengen bis zu 4,0 dz/ha gegeben wurden, steigen doch Ertrag-N-Prozente und absolute N-Mengen bis zur Höchstgabe von 4,0 dz/ha kontinuierlich an.

Weiterhin ist die gute Übereinstimmung zwischen den jeweils gefundenen und berechneten Werten hervorzuheben, wodurch die gefundenen Ergebnisse einen hohen Grad an Sicherheit erhalten. Was aber unsere Fragestellung anbetrifft, so wollen wir auf drei wichtige Punkte unserer Beweisführung hinweisen:

1.) Bis zum letzten Ertragsanstieg — sowohl bei den Wurzeln, wie bei den Blättern — steigt auch in gleicher Weise der prozentische N-Gehalt weiterhin an. Nach dem „Umkehrbaren Ertrags-Stickstoff-Gesetz“ dürfte das auf keinen Fall eintreten! Führt man hier für den höchsten Ertrag die Willcox'sche Berechnung durch, so muss bei 4 dz ha N-Düngung ein prozentischer N-Gehalt von Wurzeln + Blatt in Höhe von 2,29% in Rechnung gestellt werden. Wir würden dann für $Q = (356/2,29)$ einen Wert von 156 erhalten. Mit anderen Worten heisst das, dass der Höchstertrag (Wurzel + Blatt) nach Willcox bei 156 dz/ha liegen muss. Zieht man hiervon die Blattmengen ab, so ergibt sich bei einem Blatt: Wurzelverhältnis von 1 : 3,84 ein reiner Rübenерtrag von 115 dz/ha.

2.) Wie aus der Rubrik 17, bzw. 18 zu entnehmen ist, wurde von den Gesamtpflanzen maximal 3,30 dz/ha Stickstoff aufgenommen. Nach Willcox könnten es im Höchstoffalle 3,56 dz ha sein. Auch hier ist die Willcox'sche Annahme nicht zutreffend, denn wir können hier eine Gleichung ableiten (siehe Gleichung 18, wonach in unserem Falle 8,0 dz/ha Stickstoff aufgenommen werden können.

Im Feldversuche werden solche klaren Beziehungen nie, oder nur ganz selten zu erzielen sein, weil hier der verstärkten Anwendung N-haltiger Düngemittel enge Grenzen gesetzt sind, die nicht zuletzt durch den Wachstumsfaktor Wasser bestimmt werden. Aber auch da hat man mit gutem Erfolg einen Ausweg gefunden. Wir meinen die Anwendung erhöhter N-gaben durch zeitliche Staffelung. Wenn es nämlich gelingt, den Stickstoff den Pflanzen entsprechend ihrem Wachstumsrhythmus anzubieten, kommen wir den von Mitscherlich geforderten N-Gaben zur Erzielung von Höchsterträgen, schon bedeutend näher.

In diesem Zusammenhang sei auf die grundlegenden Arbeiten von Selke⁶⁾ verwiesen, die klar zeigten, dass durch erhöhte und zeitlich differenzierte N-gaben zwar kein sichtbarer Ertragsanstieg zu verzeichnen war, wohl aber eine Erhöhung des prozenti-

schen N-Gehaltes der betreffenden Pflanzen, sowie eine Qualitätsverbesserung.

So konnte erst kürzlich *Primost*⁷⁾ weit günstigere Ergebnisse bei Kartoffeln erzielen. Bei extrem hohen Gaben an P_2O_5 und K_2O als Grunddüngung, steigerte er die N-Düngung bis zu Gaben von 170 kg/ha. (Die Unterteilung der N-Gaben interessiert hier weiter nicht).

TABELLE IV

Knollenerträge der Speisekartoffel „Ackersegen“			
	Parzelle kg/ha N	Knollenertrag in dz/ha	Prozentischer N-Gehalt
1	50	323	0,27
2	80	379	0,30
3	120	392	0,32
4	170	417	0,34

Hier sind dieselben klaren Beziehungen zwischen Ertrag und Stickstoffprozent festzustellen. (Leider fehlen die entsprechenden Angaben beim Kartoffelkraut, sodass eine weitere Berechnung unterbleiben muss!). *Willcox*³⁾ selbst gibt einen durchschnittlichen prozentischen N-Gehalt von 0,70% an, sodass dann Q (also der maximale Ertrag) bei $(356/0,70) = 509$ dz/ha liegen würde. Zieht man hiervon noch $1/3$ für das Kartoffelkraut ab, so würde sich ein maximaler Knollenertrag von 339 dz/ha ergeben; 417 dz/ha wurden aber bereits geerntet!

Aus diesen beiden Beispielen geht klar hervor, dass durch diese willkürliche Definition jeder x -beliebige Höchstertrag angenommen werden kann, je nachdem, welcher prozentische N-Gehalt zu Grunde gelegt wird.

Da *Willcox* das *Mitscherlich'sche Wirkungsgesetz* der Wachstumsfaktoren grundsätzlich und im Prinzip anerkennt (und lediglich andere Schlussfolgerungen daraus ableitet), können mit demselben Recht Gefäß-Düngungsversuche als weiteres Beweismaterial herangezogen werden. Auch hier ist es durchaus korrekt und erlaubt, Q für Gefäße zu berechnen.

Ein Gefäßversuch — ebenfalls von *Mitscherlich* durchgeführt — zeigt folgende Abhängigkeit zwischen Ertrag und prozentischem N-Gehalt. (Versuchspflanze Hafer in reinem Hohenbocker Quarzsand. P_2O_5 , K_2O und CaO ausreichend⁸⁾).

TABELLE V

Düngung, g Amm. Nitrat	Ertrag g	% N	mg N
0	10,6	0,59	62
0,12	28,8	0,51	147
0,30	50,1	0,54	269
0,80	111	0,61	673
1,20	140	0,70	973
1,60	181	0,75	1351
2,00	189	0,86	1622
2,40	203	0,96	1941
4,00	206	1,40	2885

Wenn man hier analog zur „Agrobiologischen Stickstoffkonstanten“ des Feldes x_N (Feld) = 356, eine Konstante für Gefäßversuche berechnet, so würde x_N (Gefäß) unter Zugrundelegung von $c = 0,122$ nach Willcox =

$$x_N \text{ (Gefäß)} = \frac{2 - \log (100 - y)}{c} = 109 \text{ je g/Gefäß}$$

betragen. Q würde dann bei einer Düngung von 4 g Ammonnitrat = $109 : 1,40 = 78$; bei einer Düngung von 1,2 g dagegen = $109 : 0,70 = 156$ werden; also auch dann liegt noch der bei höchsten Stickstoffgaben tatsächlich gefundene Ertrag weit über dem nach Willcox berechneten.

TABELLE VI

Oberirdische Pflanzentrockensubstanz									
Behandlung Stickstoff	Senf			Malve			Serradella		
	Ertrag g	N %	ab- solut	Ertrag g	N %	ab- solut	Ertrag g	N %	ab- solut
2,0 g in einer Gabe	86,4	1,07	0,92	11,0	1,25	0,13	27,2	2,50	0,68
0,1 %	68,5	2,73	1,90	69,1	3,41	2,36	59,0	3,88	2,29
0,2 %	112	3,42	3,83	112	4,79	5,35	75,5	3,47	2,71
0,4 %	81,3	5,26	4,25	44,0	5,91	2,60	57,1	5,23	2,99

Bekanntlich erfolgt die idealste Nährstoffversorgung unserer Pflanzen mit Hilfe sogenannter Nährlösungen. Auch dafür ein Beispiel: In Mitscherlich-Gefäßen wurden zu Hohenbockaer Quarzsand eine Grunddüngung von 1,5 g K_2O , 1,5 g P_2O_5 und 1,5 g $CaCO_3$ gegeben. Stickstoff wurde als Ammonnitrat in der

täglichen Giesslösung verabreicht, und zwar in Konzentrationen von 0,1% — 0,2% und 0,4%-iger Lösung. Zum Vergleich diene eine Versuchsreihe, bei der 2,0 g N als Ammonnitrat in der üblichen Weise beim Ansetzen der Versuche verabfolgt worden war.

Die Ergebnisse zeigen an Bemerkenswertem: Einmal das stetige Ansteigen der N % innerhalb der Nährlösungsreihen bis zur 0,4%-igen Lösung. Dadurch wird es uns aber ermöglicht, den prozentischen N-Gehalt verschiedener Pflanzen bis auf eine Höhe zu steigern, die im heutigen feldmässigen Anbau noch lange nicht erreicht wird. Zum Zweiten fällt — ganz allgemein — der starke Unterschied im prozentischen N-Gehalt zwischen Nährlösungsbehandlung und Vorratsdüngung (2,0 g) auf, und drittens ist sehr bemerkenswert, dass durch die Nährlösungsbehandlung die normalerweise bestehende charakteristischen Unterschiede im prozentischen N-Gehalt zwischen Nichtleguminosen und Leguminosen vollständig beseitigt wurden.

Wendet man auch hier die Willcox'sche Berechnungsweise an, $Q = 109/N\%$, so ergibt sich, dass die Höchsterträge der drei Versuchspflanzen um 20 g/Gefäss liegen müssten! Sie erreichen aber bereits eine Höhe von 44 bis 81 g.

Zusammenfassend kann über das „Umkehrbare Ertrags-Stickstoff-Gesetz“ (Inverse Yield-Nitrogen Law) von O. W. Willcox festgestellt werden:

1.) Die Grundlagen des „Umkehrbaren Ertrags-Stickstoff-Gesetzes“ sind weder in ihrer Gesamtheit, noch in einzelnen Teilen vertretbar, weil sie die mathematische Formulierung des Wirkungsgesetzes der Wachstumsfaktoren in einer Weise auslegen, die zu Trugschlüssen führen müssen.

$$\left(x_N = \frac{2 - \log(100 - 100)}{c} = 356, \text{ statt } = + \infty \right)$$

2.) Die Beziehungen, wie sie nach Willcox zwischen prozentischem N-Gehalt und dem Ertrage einer jeden Pflanze bestehen sollen, existieren nicht!

3.) Eine Fixierung der sogenannten „Agrobiologischen Stickstoffkonstanten“ $Q = 356$ ist unmöglich, und widerspricht den Erkenntnissen der Pflanzenphysiologie, da von allen Wachstumsfaktoren nur ein einziger, der Stickstoff, Berücksichtigung findet.

4.) Im angeführten Düngungsbeispiel Seite 330 zeigte sich, dass

hier im Höchsthalle 8 dz/ha N, und nicht nur 3,56 dz/ha von den Pflanzen aufgenommen werden können.

5.) Damit entfällt aber auch jeder Einwand von Willcox, den er gegen die Erhöhung des Wirkungswertes des Stickstoffes von $c = 0,122$ auf $c = 0,2$ anführt.

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BIOSYNTHESIS OF AMINO ACIDS ACCORDING TO SOIL FERTILITY

III. BIOASSAYS OF FORAGE AND GRAIN FERTILIZED WITH "TRACE" ELEMENTS *)

by FRED E. KOEHLER and WILLIAM A. ALBRECHT **)

That fertilization of the soil brings about improvement in the nutritional values of feed as measured by animal growths, was reported by Albrecht and Smith^{1) 8)}; McLean³⁾; and Kendall, Nevens and Overman²⁾. When the problem of nutrition points to the proteins, not only in totals measured by nitrogen but also in the proper balance of their constituent essential amino acids, then, the synthesis of these nitrogenous compounds by plants (and microbes) for higher nutritional values in forages and grains for animals may well be considered as a responsibility of proper soil management. That the concentrations of various amino acids in plants are influenced by the different inorganic nutrient elements and the ratios of their concentrations in the medium in which the plants are grown was shown by Sheldon, Blue, and Albrecht^{5) 6) 7)}. The work reported here was another attempt to study this relation of the amino acid array in a forage (alfalfa, or lucerne) and in a grain (corn or maize) to the soil treated with "trace" as well as major elements-of fertility values. It was an attempt, also to make bioassays of the forage and the grain by means of rabbits with even amino acid- and other nutritional-supplements in the rations for these test animals.

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EXPERIMENTAL METHODS

The forage crop of alfalfa (lucerne) was grown on a residual limestone soil (Newtonia silt loam) in Southwest Missouri, (U.S.A.), given four soil treatments. These were: (1) magnesium sulfate (100 pounds acre) plus a trace element mixture at the same rate; (2) magnesium sulfate only; (3) no treatment; and (4) trace elements (100 pounds acre). The mixture of the trace elements contained manganese sulfate, 55 pts; copper sulfate, 20 pts; borax (sodium tetraborate), 10 pts; zinc sulfate, 10 pts; and cobalt sulfate, 5 pts.

Chemical analyses of the samples of four alfalfa hays grown on the soils given these different treatments were made for the elements nitrogen, calcium, magnesium, phosphorus, potassium, sodium, and copper. The hays were subjected to microbiological analyses for nine of the ten amino acids essential for the white rat (phenyl alanine was not determined). They were then subjected to a bio-assay of their nutritional values by feeding them to four groups of young, white, New Zealand rabbits. Some of the rabbits were fed *ad libitum* and some were *pair fed*. All of them were given a supplement of ten grams of oats per head per day.

The grain crop of corn (maize) was grown on one of the planosols (Cherokee silt loam) also in Southwest Missouri (U.S.A.) in a test using each of seven different combinations of the five major elements (nitrogen, phosphorus, potassium, calcium, and magnesium) as fertilizers in conjunction with each of three rates, viz. 0, 100, and 300 pounds per acre of the trace element mixture. Tests were made of (a) the hardness of the grains on grinding, (b) the rabbits' preference for them in lots of seven, (c) the amounts of each of the ten essential amino acids they contained according to microbiological assays, and (d) nutritional values by feeding some of them from different soil treatments to rabbits, with and without supplements of the commonly deficient three amino acids, viz. lysine, tryptophane, and methionine, and of vitamins and casein.

RESULTS FROM TRIALS OF THE FORAGE

In the chemical studies of the alfalfa (lucerne) hays, the major differences in their relation to the fertility treatments of the soil growing them were found for the concentrations of phosphorus

and for some of the amino acids. Although the concentrations of total nitrogen in the alfalfa varied but little with the soil treatments, the concentrations of the tryptophane and the methionine in the hay were increased by the addition of magnesium and trace elements in combination (Table 1). The fact that these two amino acids are most commonly deficient in many forages gives particular significance to this discovery. When fed the hays grown on the soil treated with magnesium plus the trace elements, the rabbits made better gains by 15 per cent than those by the rabbits fed the hay grown with no soil treatments (Table 2). Twenty-two per cent less hay was required per unit of gain in the former case than in the latter (Table 2).

TABLE I

Concentrations of nitrogen, phosphorus, and two amino acids in alfalfa hay according to treatments of the soil growing it				
Treatments of the Soil	Concentrations			
	Nitrogen (%)	Phosphorus (%)	Tryptophane ¹ (mg/g)	Methionine (mg/g)
Magnesium and trace elements	3.05	0.247	2.52	5.44
Magnesium	3.20	0.224	—	—
No treatment	3.12	0.218	1.86	4.57
Trace elements	3.19	0.211	—	4.20

TABLE II

Gains in weights and alfalfa hays consumed per gram of gain by rabbits according as hays fed them were grown on soils given different treatments						
	Gains (G/Animal)			Hay		
	ad lib. Feed	Paired Feed	Av.	Offered G/Animal	Wasted G/An.	Consumed G/G Gain
Magnesium and trace elements	879	809	849	4536	1105	4.04
Magnesium	742	748	744	5050	1561	4.69
No treatment	751	711	740	5084	1491	4.86
Trace elements	684	723	699	4840	1420	4.89

It is significant that the forage grown on the soil given the treatments consisting of magnesium alone, or of only the mixture of the five trace elements (manganese, copper, boron, zinc, and cobalt), gave (a) no significant increase in the concentrations of the commonly deficient amino acids in the forage; (b) no greater gains in rabbit weights; and (c) no significantly greater gains per

unit of feed consumed than those from the forage grown on the soil given no treatment. However, when two of these treatments of the soil were combined, i.e. applying the magnesium along with the five trace elements, then the forage was improved decidedly (a) in its concentrations of tryptophane and methionine, (b) in the gains in the weights of rabbits in a given time, (c) and in the gains per unit of feed consumed.

What this all would mean in terms of the fecundity of the rabbits was not studied. It suggests, however, that research in plant nutrition would be more remunerative if, instead of using each element singly as a soil treatment and then these in various combinations of a few of the essential elements, all the known elements were applied in combination and then in successive trials an individual one, or combinations of them, were withheld in the manner similar to that used in studies in human nutrition testing the amino acids (4). It suggests still further, that the soil treatments can improve the nutritional values of the forages for animals, and that these improvements probably come via the better balance of the amino acids and other complex compounds of synthetic origin within the plants.

RESULTS FROM TRIALS WITH THE GRAIN

While, naturally, one cannot know how corn tastes to a rabbit and cannot design methods of measuring the differences in tastes, it seemed that possibly the hardness of the corn grain might be a factor in the rabbits' preference for that from one soil treatment over another when their selection from a lot was permitted. Accordingly, the respective hardnesses of the samples of corn from the twenty-one different soil treatments were determined by running each through a Wiley laboratory mill and sieving it with a nest of 10-, 40-, and 80-mesh sieves to find the percentages of the samples passing through each.

There was the least variation in hardness of the grain for the seven combinations of the major nutrient elements without additions of the trace elements as soil treatments. For this lot from 14.7 to 21.3 per cent passed through the 10-mesh sieve. But when 100 and 300 pounds, or increasing amounts, of trace elements supplemented the major nutrient elements applied to the soil, then there was

greater variation in the hardness of the grains, namely, from 14.3 to 30.7 and from 8.1 to 33.7 per cent, respectively, passing the 10-mesh sieve.

But when these samples of grain were submitted to the rabbits in lots according to the seven major nutrient elements as the only variable while the soil treatment with trace elements was a constant, the variations in the amounts consumed showed the maximum taken over the minimum amounting to 1.25 times for zero trace elements, 2.3 times for 100 pounds of these applied to the soil, and 2.05 times for their application at 300 pounds per acre in combination with the major nutrients. That there was no correlation between the hardness as measured by grinding and the selection by the rabbits as shown by amounts consumed in these lots, was clearly demonstrated by the fact that these animals consumed exactly the same amounts of the hardest and of the softest of the samples. Surely, then, the differentiation by the rabbits between these grains was not according to their hardness as measured by the extra labor of grinding or mastication, but was according to the other differences invoked by differences in the soil treatments.

It was significant that as an average, in this preference testing of the lots of grain when the major nutrients as soil treatments varied with a single trace element treatment in combination, the amounts of grain consumed as a lot were highest for the soil treatments using no trace elements, the next highest were for the 300 pounds of this treatment, and lowest for the soil treatment with 100 pounds of trace elements as supplementary fertilizer. When the heaviest applications of the trace elements to the soil (300 lbs/A) were considered, it was significant that the rabbit preference, in terms of maximum grain consumption, singled out this soil treatment of the trace elements, in some cases of it in combination with or without the major elements accompanying it. This latter choice was confirmed by field observations when the grain on the plot with only 300 pounds of trace elements was taken by rodents much more than that on any other plot, and by the fact that among the twenty-one bags in storage, the mice cut into only the bag representing this particular soil treatment of trace elements alone and in their heavy applications.

In a second trial, in which the major nutrient elements were nearly a constant soil treatment while the trace elements were the

variable and the consumption of grain represented the rabbits' choice between 0, 100, and 300 pounds of the trace elements as soil treatments, the preference by them was (a) for the corn fertilized with 100 pounds of trace elements when these three rates each were combined with only dolomite or with dolomite and starter fertilizers; (b) for no trace elements when in conjunction with most complete major nutrient fertilization; (c) for 100 pounds of these when the complete fertilization used superphosphate in place of rock phosphate; and (d) for 100 pounds of trace elements when in combination with more complete major treatments omitting potassium; but for 300 pounds of trace elements when these were used alone. As an average, the preference pointed to the 100 pounds of trace elements in these three sets of combinations according to 0, 100, and 300 pounds of trace elements.

While the selections of the grains by the rabbits, or their preferences for consumption, presented marked discriminations according to the treatments of the soil growing the grains, such preferences pointed to no particular physiological factor as possibly responsible. Consequently, the corn grains were fed to lots of rabbits (4-7 per lot) for six weeks with the corn supplemented by amounts of (a) each of the pure amino acids separately; namely, tryptophane, .11-.12 per cent by weight; methionine, .23-.30 per cent; and lysine .20 per cent, (b) vitamins *) and (c) casein 5 per cent. The gains in weight by the rabbits, and the corn required per gram of gain, as given in Table 3, show clearly that the soil treatments growing the corn exerted a decided effect on the nutritional efficiency of the grain as measured by the gains in weight of the rabbits.

Of the treatments using only the major elements on this soil, the dolomitic, or magnesium, limestone was most singular in giving corn of high nutritional efficiency when it required but 5.48 grams to make a gram of gain in weight of rabbit. The treatment next

*) With each 400 g corn there were added

5000 units — Vitamin A

1000 units — Vitamin D

2.5 mg — Vitamin B₁ (Thiamine hydrochloride)

2.5 mg — Vitamin B₂ (Riboflavin)

50.0 mg — Vitamin C (Ascorbic acid)

20.0 mg — Nicotinamide (Niacinamide)

5.0 mg — Pantothenic Acid (Calcium salt)

0.5 mg — Vitamin B₆ (Pyridoxine hydrochloride)

1.0 mcg — Vitamin B₁₂ (Cyanocobalamin)

TABLE III

No. Soil treatment	Treatment	Concentrations of total nitrogen, lysine, tryptophane and methionine in corn (maize) according to soil treatments growing it, and gains in weights by rabbits consuming it with and without supplements					Gain/Rab. G/6wks	G Corn/ G Gain
		Nitrogen %	Lysine mg/g	Tryptophane mg/g	Methionine mg/g	Supplement Fed		
1	Magnesium limestone	1.61	10.0	0.60	5.7	None	291	5.48
2	Ditto 1 plus starter fert. (a) . .	1.58	8.8	0.74	5.0	—	—	—
3	Ditto 2 plus nitrogen, pot. rock phos. and trace ele. (b) . . .	1.81	7.0	0.67	5.9	Tryptophane Methionine (c) Lysine	281	5.59
4	Ditto 3 plus extra trace ele. (d)							
5	and superphos.	1.79	10.0	0.69	4.9	None	129	13.67
6	Ditto 4	1.79	10.0	0.69	4.9	Methionine	204	6.53
7	Ditto 3 minus potassium . . .	1.72	7.0	0.56	5.7	None	175	8.28
8	Ditto 6	1.72	7.0	0.56	5.7	Tryptophane	260	5.31
9	None	1.78	7.9	0.62	5.7	None	202	7.97
10	Ditto 8	1.78	7.9	0.62	5.7	Vitamins (e)	233	6.79
11	Ditto 8 plus trace elements . .	1.62	7.9	0.59	5.4	None	212	5.72
	Ditto 10	1.62	7.9	0.59	5.4	Casein (f)	344	4.30

(a) The starter fertilizer used as the soil treatment consisted of 250 lbs per acre of a 4-12-4.

(b) The complete soil treatment consisted of the starter fertilizer, after plowing under per acre 400 lbs ammonium nitrate, 200 lbs potassium chloride, 1500 lbs rock phosphate and trace elements, 100 lbs.

(c) These three amino acids were added in pure form since in respect to the commonly considered needs for rats the corn was seriously low in these amino acids.

(d) The trace elements in total amounted to 300 lbs per acre, while other treatments were the duplicate of treatment No. 3.

(e) This was a composite of many vitamins. (See footnote page 341).

(f) Purified casein was a supplement to the extent of five per cent of the ration.

in the decreasing order of efficiency was the trace elements only, when 5.72 grams of corn were required per gram of gain. Corn grown without any treatment required almost eight grams, 7.97, to make one gram of rabbit gain. When heavy soil treatments of major elements were used omitting potassium, it required 8.28 grams of corn for one of gain. But when the major five elements, including superphosphate in place of rock phosphate, and the extra trace elements were the soil treatments growing the corn grain, it required 13.67 grams of corn per gram of gain in body weight.

That these efficiencies could be improved by the addition of (a) some of the more commonly deficient amino acids, (b) the more complete protein (casein), and (c) the vitamins, is clearly indicated by the greater nutritional efficiency of the corn when these supplements were fed along with it. That the trace elements alone and the dolomitic limestone, all not commonly considered effective for increasing the yields as bushels per acre, served to improve the nutritional efficiency is not only startling, but also significant. It suggests that we have been led astray from nutritional services as the objective in growing this grain. That the fertilizer treatments with major elements, commonly considered most beneficial for increased yields as bushels of grain per acre, should have been so inefficient in giving the grain nutritional values is even more startling. That grain so grown should compel an animal to eat 2.5 times the amount to make the gain possible when eating corn grown on the same soil treated with only dolomitic limestone, points clearly to our past management of the soil for the delivery of vegetative bulk but not necessarily for nutritional efficiency by that mass produced.

These results show that by means of bioassays of our forages and grains, we shall be able to manage our soils more judiciously to produce these feeds for efficient nutritional services, and to recognize the benefits by magnesium, by the trace elements and, by other aspects of soil fertility not yet, or only recently, given attention as beneficial additions to the soil.

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A LABORATORY STUDY OF THE DECOMPOSITION OF VEGETABLE DEBRIS IN RELATION TO THE FORMATION OF RAW HUMUS

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INTRODUCTION

Müller¹⁰⁾ 1878-1884, 1887, tended to regard different humus types as the products of different chains of biochemical reactions and to be independent of the rate of litter decomposition.

This early view point became overlooked and most writers up to about 1930 wrote of "the rate" of decomposition as conditioning the type of humus to be derived from a particular type of litter. They attempted to explain the formation of raw humus from conifer needles or the leaves of ericaceous shrubs as being due to the slowing up of microbiological activity or to the inhibitory effect of toxic substances such as ethereal oils (von Koch⁷⁾) or to the presence of resistant substances, such as lignin (Grosskopf³⁾) or cutin, or to unfavourable physical factors such as low temperature, shortage of water, or poor aeration (Ramann *et alia*).

These theories of raw humus formation were supported by men such as Ramann and Melin⁸⁾. Ramann in the first edition of his textbook wrote "a duff results when conditions unfavourable to decay and putrefaction predominate", but later altered it to read "a duff results when conditions are unfavourable to decay".

Melin⁸⁾ in 1930 carried out experiments in which he tried to correlate loss in weight and carbon dioxide production of litter with factors such as calcium content, nitrogen content, etc., and he obviously believed that humus accumulation was due to a slow rate of decomposition since he stated that "The rapidity with which dead residues of vegetation decompose exercises a decisive influence upon the character of the humus cover". Hesselmann⁵⁾ in 1926 had expressed a similar opinion.

Romell¹¹⁾ (1932) disagreed with the contemporary views considering them to be too preoccupied with considerations of the effects of purely chemical factors upon the rate of decomposition. He surveyed critically the European work on the subject by such investigators as Vater¹³⁾ and Tamm¹²⁾, who investigated a Brown Forest Soil with crumb mull which he found to contain 28.7 kg of organic matter per cubic metre and a strong mor forming and podsolizing site which contained 27.9 kg per cubic metre. Romell¹¹⁾ came to the conclusion that there existed no evidence for believing the total weight of organic matter in and on a mor soil to be any greater than that in and on a mull soil. Further, he surveyed the work which had been done on respiration of the soil and although the data were not strictly comparable he came to the conclusion that there existed no significant evidence pointing to a greater respiration rate on mull than on mor sites. These findings were at variance with the current views so he carried out some experiments of his own in New York State and found that while the respiration of a strongly mor forming soil under White Pine was approximately the same as that of a mull site under mixed hardwoods, the total weight of organic matter in the mull soil was twice that in the mor, despite the fact that the forest floor of the former site contained nothing but a few dead leaves, while that of the latter possessed a thick mat of raw humus. In this case the respiration rate of the mor was twice that of the mull, weight for weight.

Romell¹¹⁾ further developed his biological theories (1935, 1938) stressing the importance of the acidic nature of fungal decomposition products in the formation of mor and of nitrification in reducing acidity by enabling acid utilising organisms to exist and multiply. He reviewed the silvicultural practices designed to foster the production of mull from raw humus and suggested that as the result of thinnings and cultivation, the physical environment of the forest floor was relatively little changed but that the death of roots in the humus layers, the mechanical disturbance of fungal hyphae and the mixing of inorganic ions with the unincorporated humus might create conditions more favourable for the growth of mull forming organisms. Romell¹¹⁾ thus regarded the chemical fertility of the environment as being indirectly important to humus formation by determining the type of micro-organic population, but he left unanswered the question whether an improvement in

physical factors alone can initiate a mull forming reaction in a raw humus, and unproven the question whether the rate of decomposition determines the type of humus formed.

Wittich¹⁴⁾ infers that the rate of litter decomposition is not the sole criterion of mull formation "with regard also to the breakdown of raw humus it is important that it should be so directed that at the same time valuable mild humus, linked to minerals, should be produced". But, thereafter he refers consistently to the rate of litter decomposition as being the overriding factor in the type of humus to be produced. He points out the reciprocal relationship between soil and litter, and shows that the greater the mor forming tendency of a species the richer in bases must the soil be in order that a mull may be produced; and further that the chemical content of leaves depends not only upon the species but also upon the soil. He appears in general to owe more to Ramann than to Romell.

Romell's¹¹⁾ views were challenged in 1939 by Broadfoot and Pierre¹⁾ who were of the opinion that the overriding factor governing the type of humus produced is the rate of decomposition of the litter, this decomposition rate depending, according to them, largely upon the inorganic fraction of the dead leaves.

The work of Romell¹¹⁾ in investigating respiration rates and humus weight *in vivo* is open to some criticism. The intimate humus present in a mull soil probably represents the accumulation of many years incorporation and is not appreciably reduced by fire, whereas the raw humus with which it is compared is largely unincorporated into the mineral soil and could probably represent the accumulation of a much shorter period of time, in fact since the last forest fire. Furthermore, Romell¹¹⁾ measured the total respiration of a unit area of mull soil and compared it with the total respiration of a corresponding area of raw humus. He therefore failed to distinguish between the respiration rates of material in different stages of decomposition, and between living and dead plant material.

From this brief survey of the subject it appears that two apparently elementary questions remain incompletely answered:

- (1) Does the rate of litter decomposition determine the type of humus produced?
- (2) Can physical factors alone stimulate a change from mor to mull?

An investigation into chemical or biological processes is outside the scope of this enquiry which is concerned to supply evidence, mainly quantitative, which may help to answer these two questions.

THE MUTUAL INFLUENCE OF THE LITTER OF VARIOUS SPECIES
ON RATE OF DECOMPOSITION

(a) *Experimental Principles*

With the exception of Romell¹¹⁾, previous workers have planned their experiments assuming that a high carbon dioxide production rate or a rapid weight loss are closely correlated with mull-forming properties, and have based their conclusions on this assumption. Such an assumption appears to the writer, however, to be unwarranted. For example, Melin⁸⁾ in 1930 carried out experiments on pure material with the idea of correlating known raw humus forming species, such as pine, with a low rate of decomposition. If, however, mull-forming ability and incorporation are independent of the rate of decomposition, information gained by working with pure samples may be misleading since it may by chance happen that the mull forming species under investigation is slower than the mull forming species chosen for comparison, but faster than some other mull forming species not investigated.

So far no precise quantitative experiments on the rate of litter breakdown have been carried out over a reasonably long period of time. But different stages of decomposition having different carbon dioxide production rates may occur at different times of the year with different species. Therefore, any investigation should extend ideally over a period of at least twelve months.

The first two experiments were planned with the idea of investigating the effect upon the weight loss and respiration, as indicated by oxygen absorption, of Spruce, of a factor believed empirically to promote the formation of a mull humus from Spruce needles, namely, the addition in different proportions of various broad leaf tree and herb litter.

The hypothesis is often advanced that admixture of hardwood litter with Spruce litter speeds up the apparent decomposition in the forest. If this is due to the hardwood litter speeding up the rate of decomposition the percentage loss in weight of a mixture of the two species should be greater than the mean of the percentage

losses of pure samples of each of the constituents since the mull-forming decomposition phase of Spruce will be correlated with more rapid decomposition. If, however, such an admixture affects not only the rate, but the type, of decomposition, the mixture might either lose more or less weight than each of the constituents, depending upon whether or not the mull-forming reaction of Spruce needles leads to a more rapid or a less rapid respiration than does the mor-forming reaction.

(b) *Collection and preparation of material*

It has been shown, Wittich *et alia*¹⁴), that the chemical content of the leaves of a particular species varies with the site. It was necessary, therefore, to collect all the material from a site as nearly uniform as possible. Furthermore, it had to be a site on which Spruce forms a raw humus while the other litter forms a mull. The Plateau Gravel in Bagley Wood was the most suitable area near Oxford and this site was accordingly chosen.

All the material was, of necessity, gathered in February. This is not considered to be a disadvantage, however, since much of the water soluble material had by then been removed, and this material being very easily decomposed is probably of little importance in deciding the ultimate resistance to decomposition and may in fact mask any such tendency should it exist.

Material of the following species was collected from the ground. In each case the sample included litter from at least fifteen different individuals.

Species	Form	Average Age	Humus
<i>Picea abies</i> L. Karst. . . .	Pure stand	45 years	L. F. & H. layers with surface leaching
<i>Pinus radiata</i> D. Don. . . .	Pure stand	45 years	" " "
<i>Carpinus betulus</i> L. . . .	Pure stand	40 years	Mull
<i>Corylus avellana</i> L. . . .	Scattered trees		Mull
<i>Quercus borealis</i> Michx. . .	Scattered trees	45 years	Mull
<i>Epilobium angustifolium</i> L.	Leaves were picked from dead stems		

The litter was air dried. The only Spruce needles used were those showing no external signs of decomposition visible to the naked eye and these were picked out by hand. In order to facilitate weighing and handling, the leaves were coarsely ground and the pine needles cut into 1½ inch lengths. The Spruce needles were left untouched.

Of this material part was used for determinations of weight loss alone and part for the periodic determination of respiration rate as well as weight loss.

Variations in weight of more than .001 g in 2 g were found to result from variations in atmospheric humidity. In order to ensure weighings reliable to the third place of decimals, the material was allowed to reach equilibrium with the atmosphere and each complete experimental series weighed out on a dry day. On each day that any particular species of litter was weighed two extra samples were weighed, oven dried and weighed again in order to arrive at the oven dry weight of the experimental samples. The percentage loss in weight of the two samples never differed by more than 0.5%.

EXPERIMENT 1

INVESTIGATION OF WEIGHT LOSSES

Approximately two grams of material were weighed out into each of 50 two inch petri dishes forming 10 series, as follows:

Series	No. of dishes	Species & range
1	7	100% <i>Picea abies</i> to 100% <i>Quercus borealis</i>
2	6	90% " " " 100% <i>Carpinus betulus</i>
3	6	90% " " " 100% <i>Epilobium angustifolium</i>
4	4	75% " " " 100% <i>Corylus avellana</i>
5	4	75% " " " 100% <i>Pinus radiata</i>
6	7	100% " " " 100% <i>Quercus borealis</i>
7	4	90% " " " 100% <i>Carpinus betulus</i>
8	4	90% " " " 100% <i>Epilobium angustifolium</i>
9	4	90% " " " 100% <i>Corylus avellana</i>
10	4	90% " " " Ash obtained from weight of <i>Quercus borealis</i> equivalent to 75%.

Each dish of litter in series 1-5 was inoculated with .01 g of soil (.005 g mineral soil) taken from a number of places in a zone about one inch deep between the top of the mineral soil and the bottom of the overlying litter under a stand of mixed Red Oak, Larch and Pine on the Plateau Gravels, (Melin⁸). At the same time the samples were moistened with distilled water for the first time.

Series 6-10 were first autoclaved and then inoculated and moistened in the same way as was the non-autoclaved group.

The dishes were spaced out in two wooden trays, each about 18 inches square, and two inches deep, and maintained at ordinary room temperature.

In order to prevent excessive evaporation and the entry of dust, each tray was covered with a sheet of glass raised by a slice of cork at each corner of the tray and surrounded by a paper margin.

The dishes were watered with distilled water twice a week, sufficient water being added to maintain the material in a pulpy soggy condition. The distilled water was taken from a reserve bottle left standing with the stopper removed in order that its contents might reach a state of equilibrium with the atmospheric gases.

The dishes were rearranged within the trays about once a month.

After five months a number of dipterous larvae were noticed in some of the dishes (they appeared to show no specific preference apart from avoiding pure Spruce and Pine). In order to prevent their escape each dish was covered with a watch glass and a small crack was left between the edge of the dish and the edge of the watch glass to allow gaseous exchange to take place.

The material was oven dried and weighed after a period of 10 months.

Results of Experiment 1.

The results are plotted in graphs 1-5. The effect of autoclaving has been, with all species excepting *Epilobium angustifolium*, to reduce the loss in weight and to render the results rather more erratic. This is of experimental interest, but the explanation is outside the scope of the present inquiry.

A straight line relationship exists between rate of breakdown and specific composition in the following cases: —

Spruce — Hornbeam

Spruce — *Epilobium*

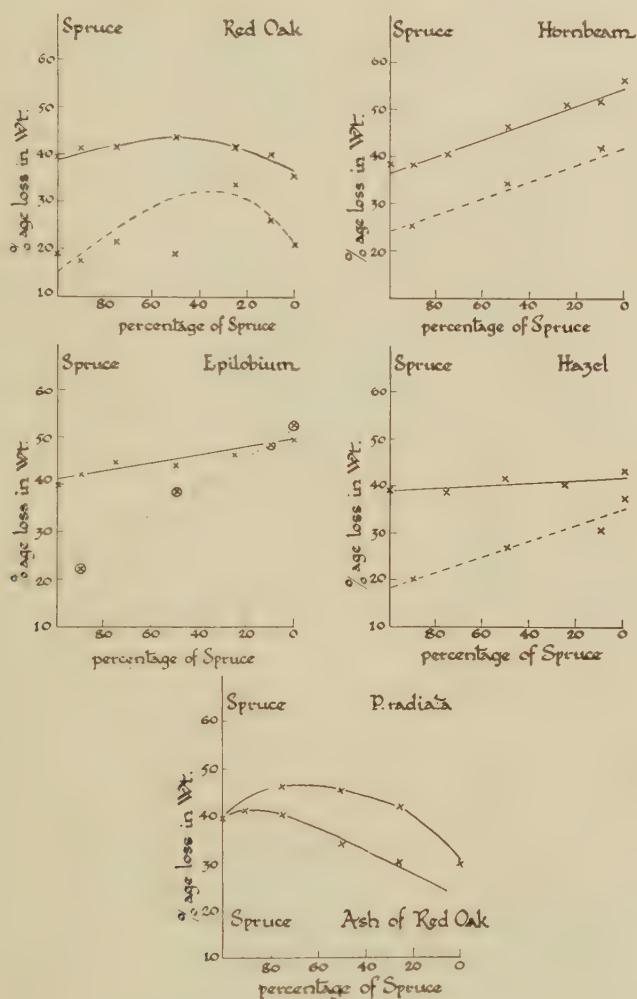
Spruce — Hazel

This suggests that under the conditions of the experiment the presence of Hornbeam, *Epilobium* or Hazel litter has no effect upon the rate of decomposition of Spruce needles. But the figures given represent weight loss and not total molecular transformation and it is not possible to say if the qualitative breakdown of Spruce needles has been modified by the presence of the hardwood litter. This seems to be unlikely since it is probable that a change in chemical processes would be reflected by a change in weight loss, representing a new energy relationship. It may be, however, that a large concentration of hardwood litter does influence the decom-

position rate of Spruce but that the absolute variations remain within the limits of experimental error and are not indicated by the graphs. While there is a significant difference between the rate of decomposition of Spruce and Hornbeam and to a lesser extent between Spruce and *Epilobium*, the absolute differences are not very great. Thus Spruce lost 39.5%, Hornbeam 56%, Red Oak 36%, *Epilobium* 49%, and Hazel 42%, by weight. There is no correlation so far as hardwoods are concerned, between rate of decomposition and the rapidity of disappearance as observed in the forest and if the experimental results could be applied to field conditions they would suggest very strongly that the rate at which litter decomposes is largely incidental to its tendency, either to form mull humus or to accumulate on the surface of the soil in the form of raw humus.

The graphs for Spruce and Red Oak and Spruce and *Pinus radiata* suggest that some sort of mutual reaction has occurred because the rate of breakdown of mixtures of the two species is greater than the rates of breakdown of the two constituents. These results must be interpreted cautiously because in the case of Spruce and *Pinus radiata* the five readings are insufficient to permit of a statistical analysis and in the case of Spruce and Red Oak the greatest absolute increase in weight loss which occurs with a 50/50 mixture is of the order of only 5-6%. The data from the autoclaved material of Spruce and Red Oak is less satisfactory and a somewhat subjective curve has been drawn based largely on the assumption that the low reading from the 50/50 mixture may represent a failure of the inoculum, but in view of the unsatisfactory nature of the data, little, if any, significance can be attached to this graph. The experiment as a whole, however, provides some scanty evidence that Spruce and Red Oak and Spruce and *Pinus radiata* litters may react mutually or that one may promote the rate of breakdown of the other and it is suggested that this phenomenon warrants more extensive investigation. But the data give no indication whatever that the type of decomposition is affected. The increased weight loss may be due to better physical conditions influencing one or both constituents, but it may reflect a change in biochemical reactions.

The five readings relating to Spruce and ashed Red Oak leaves do not lend themselves to a statistical treatment but visual inspection



- x-x-x-x Inoculated but not autoclaved
 x--x--x Inoculated and autoclaved
 o.....o Autoclaved but not inoculated

Graphs 1-5

is sufficient to suggest that when the concentration of ash is greater than that corresponding to 20% of Red Oak litter the rate of Spruce decomposition is inversely proportional to the concentration of ash, while concentrations corresponding to less than 20% of Red

Oak have no significant effect. The inhibitory effect of the ash cannot be explained. It is possible that in the process of ashing a number of insoluble oxides is produced and that as a result an unbalanced fertiliser has been applied or it is perhaps conceivable that a mull forming reaction has been initiated which in the case of Spruce results in a slower rate of decomposition than occurs with a mor forming reaction.

The results of this experiment cannot unreservedly be applied to forest conditions. In the first place the reactions took place in a glass dish which is considerably more nutrient deficient than the poorest tree bearing soil. Physical conditions of temperature and moisture were more favourable than those in the forest. Apart from a brief period during which some dipterous larvae appeared animals were excluded and, a very important point, there was little opportunity for succession, in the ecological sense, of micro-organisms, as the properties of the substrate underwent a gradual change. This last difficulty was only imperfectly alleviated by a second inoculation after the experiment had been going for about six months.

EXPERIMENT 2

INVESTIGATION OF RESPIRATION BY MEASUREMENT OF OXYGEN ABSORPTION

It was desired to trace the rates of respiration as indicated by oxygen absorption for a range of mixtures of decomposing litter over a period of time. It was necessary to be able to absorb the CO_2 evolved and to remove it without contaminating the material. Furthermore, since the evolution of CO_2 would be continuous this would have to be absorbed rapidly in order to measure accurately the amount of oxygen absorbed during a particular period of time. No apparatus could be found which was suitable for this task. Accordingly, a piece of apparatus was designed to meet these requirements. It consists of an elongated and basally flattened tubular bottle with two ground glass necks, one on the top which connects with a manometer, and one at the end through which is inserted the CO_2 absorbing device. This consists of a hollow glass tube sealed on to a glass stopper. The tube has a longitudinal

segment cut from it and this exposes about 2 square cm of potassium hydroxide impregnated filter paper (see fig. 1).

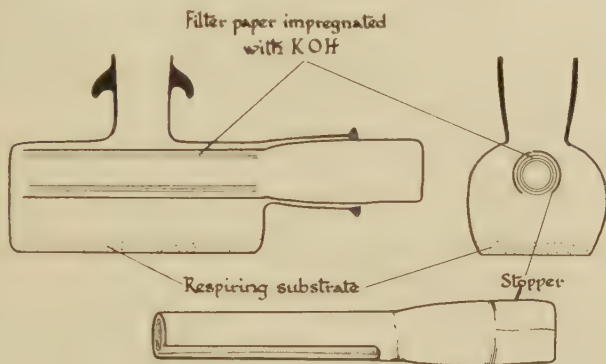


Fig. 1.

The tube is adjusted so that the exposed paper faces downwards and lies horizontally just above the surface of the respiring litter in which position absorption is rapid. The glass tube may be easily and rapidly inserted or withdrawn and effectively prevents any contamination by potassium hydroxide of the interior of the bottle. For determinations of oxygen absorption the bottle was connected to a manometer and immersed in a thermostatically controlled bath.

The manometers used in the experiment were calibrated by the chemical method of Gibson²⁾.

It was found that the equilibrium period of 30 minutes suggested by Gibson²⁾ was insufficient, and led to appreciable errors. Therefore, in all determinations the bottles were immersed in the water bath for 60 minutes before closing the manometer taps.

The CO₂ was absorbed by 10 drops of 20% potassium hydroxide. The absorbing capacity of this reagent was tested by measuring the respiration of an actively respiring sample over a period of four hours. Readings were taken every 30 minutes and the results plotted. The graph obtained by joining the points was in the form of a straight line and from this information it was assumed that the absorbing capacity of this amount of alkali was adequate.

Approximately one gram of material was weighed into each of thirteen bottles in the following proportions: —

Bottle No.	Percentages		Bottle No.	Percentages	
	Spruce	Red Oak		Spruce	Red Oak
6 and 13	100		12	40	60
1	90	10	3	30	70
10	80	20	2	20	80
9	70	30	7	10	90
4	60	40	8 and 11		100
5	50	50			

Each bottle was inoculated and watered in the manner described in experiment 1.

Bottles 11 and 13 were prepared about four weeks after the other eleven, not being previously available.

The rate of oxygen absorption was determined about once every nine days from 27th June to 10th November 1951, the O_2 absorbed being measured in each case over a period of two hours at a temperature of 22°C.

At the end of this time a consideration of the results suggested that no significant relationship existed between the rate of oxygen absorption and the proportions of Spruce and Oak litter. Accordingly six bottles were removed from the experiment but investigations were continued on the remaining seven bottles.

Results of Experiment 2.

It has already been mentioned that up to 18th October, 1951 little correlation could be detected between the rate of oxygen absorption and the proportion of Spruce litter in the mixture and six bottles were then oven-dried and weighed.

The losses in weight of the six bottles removed from the experiment were as follows: —

Bottle No.	Contents	Percentage loss in dry weight after 18 weeks
1	90% Spruce 10% Red Oak	31 %
9	70% „ 30% „	26.5%
4	60% „ 40% „	32.3%
3	30% „ 70% „	29.5%
2	20% „ 80% „	23.7%
7	10% „ 90% „	24.0%

Calculations showed the correlation coefficient to be .68 ($> 10\%$). There was this a suggestion that Spruce litter had lost more weight than Red Oak litter but no synergistic effect could be detected.

Upon investigation it was found that the series of results obtained on 13th August showed a positive correlation — correlation coefficient .7998 ($>5\%$) — between the proportion of Spruce litter in the mixture and the rate of oxygen absorption.

No correlation could be demonstrated between specific composition of the material and

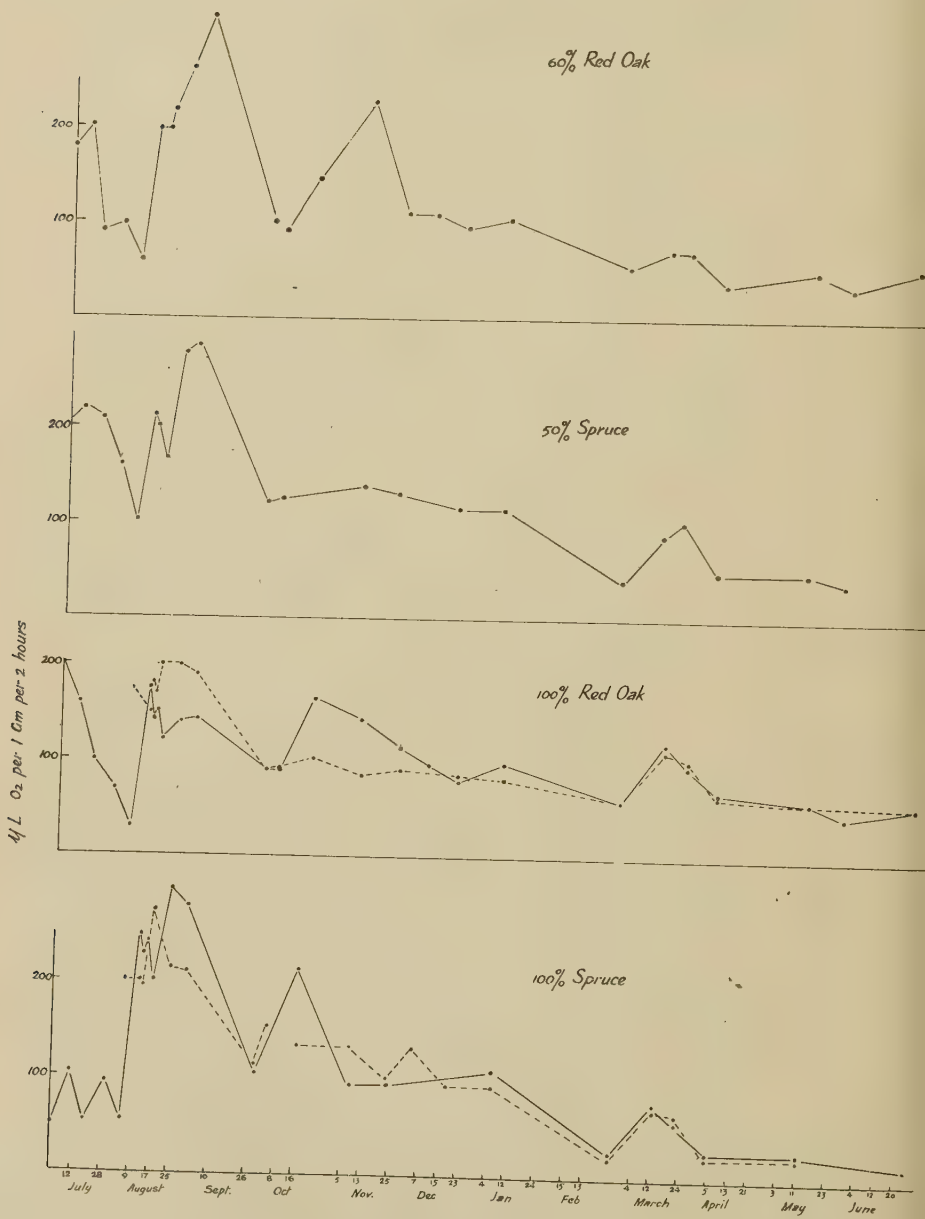
- (1) Effect of drought or water-logging
- (2) Degree or rate of recovery from water-logging
- (3) Initial respiration rate
- (4) Maximum respiration rate achieved.

Readings on the remaining seven bottles were continued until June 1952, the results of which are shown in graphs 6-9. These bear a general resemblance to each other. They are erratic but the maxima and minima occur simultaneously and there is a tendency in each case for the respiration rate to decrease with time.

The sharp fluctuations are due to the fact that the writer was away from Oxford from 22nd July to 7th August and from 3rd September to 30th September. During the first period the material was watered insufficiently and during the second period it became water-logged.

The moisture conditions between 18th August and 31st August and from 10th November 1951 until the end of the experiment in June 1952 are considered to be as near optimum as could be obtained by the method of hand watering. The effect of prolonged water-logging (probably lasting throughout September) was less decisive than that of drought. Recovery was slower, probably because it takes longer for water to evaporate than it does to add water to dry material. Two new samples, one of Red Oak and one of Spruce litter (indicated by dotted lines on the graphs) were set up on 6th August. For the first few weeks their respiration rates, since they were at a different stage of decomposition, bore little relation to those of the other samples of similar composition. But the effect of environment seems gradually to have masked the difference of four weeks in the stage of decomposition.

There was a sharp and unaccountable rise in March which cannot be explained by a rise in temperature because the bottles were, from January onwards kept not at room temperature but in an incubator at a controlled temperature of 22°C and it is believed



that the relatively small variations in moisture content from day to day were insufficient to account for such an irregularity.

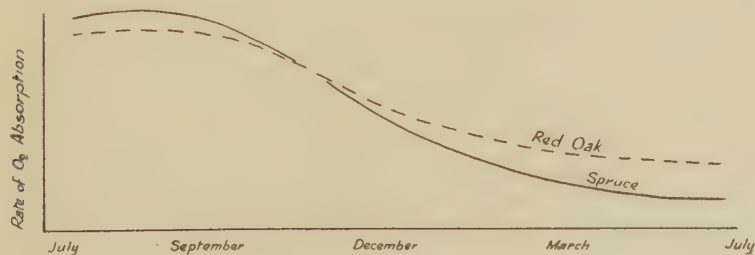
It is not possible to say precisely what stage in decomposition the two materials had reached when the experiment was started. Both Red Oak leaves and Spruce needles had been lying on the ground throughout the winter and had probably lost a large proportion of the water soluble constituents of the cells and it may well be that the material is comparable. Some idea of the main trend of decomposition may be obtained by joining together the maxima on the graphs. This is shown in a generalised form in graph 10.

The final weight losses of the bottles which had been under observation for a year were as follows:

Bottle No.	Contents	Percentage loss in dry weight after one year
6	100% Spruce	37
13	100% "	37
10	80% "	44
5	50% "	44
12	40% "	41
8	100% Red Oak	34
11	100% "	33

The measurements of oxygen absorption have revealed a difference in behaviour between the Red Oak and Spruce litters not shown by the overall weight losses.

It has already been mentioned that a positive correlation between the percentage of Spruce and the rate of oxygen absorption exists for the series of results obtained on 13th August 1951. It became obvious by about March that this tendency had been reversed and for the last few months of the experiment there was greater biological activity in the predominantly Red Oak samples than in the predominantly Spruce samples. This reversal of tendencies is illustrated in graph 10.



Graph. 10

EXPERIMENT 3

THE INFLUENCE OF ENVIRONMENT AND MINERAL
NUTRITIONAL FACTORS ON LOSS IN WEIGHT BY
MATERIAL FROM THE "H" & "B" HORIZON OF PODSOLS(a) *Experimental Principles*

Preliminary results from experiment 2 suggested that there might be no significant difference between the rates of decomposition (as measured by loss in weight) of litters breaking down to form mull and those breaking down to form mor under comparable environmental conditions. If this were so it would suggest that a change in physical conditions alone might be insufficient to stimulate the formation of mull humus from a litter already in the process of decomposing to form mor. Experiment 2 clearly showed that an improvement in water relations (provided other factors are not limiting) will accelerate the decomposition of both Spruce and Red Oak litters, the conditions being such that it is highly probable that the Spruce needles are producing a raw humus type of end product. The status of Red Oak litter is somewhat indefinite but numerous authors state that Red Oak does not form a raw humus on infertile sands and it is therefore possible that it was decomposing to form a mull type of end product in the glass dishes.

If it is the case then that the rates of decomposition of two different litters, the one probably breaking down to form a mull type and the other a mor type of end product, were influenced similarly by variations in moisture content, it is illogical to regard poor physical conditions leading to slow decomposition as being primarily responsible for the formation of raw humus. An increase in rate of breakdown may simply mean that a raw humus is being obtained more quickly. It seemed probable, however, that some information might be obtained by working with the relatively inert material representing the later stages of raw humus formation, namely "H" layer material from a raw humus layer, and "B1" horizon material from a podsol.

(b) *Material and Methods*

"H" layer material: "H" layer material was collected at a depth of about 5 inches from a number of places under a mature pine stand growing on strongly podsolized gravels (probably of

Pleistocene age) near Newbury. The subordinate vegetation consisted of scattered groups of *Pteridium* covering about 25 percent of the surface, together with occasional leggy plants of *Calluna*. The total depth of humus was about 8 inches, of which the lower 5 inches comprised the "H" layer.

The material was agitated in water and filtered through a coarse sieve to remove the larger roots. The filtrate was then repeatedly filtered through a 90 gauge sieve to remove root particles and finally allowed to settle, when a few fine particles remained floating on the surface. The residue — a dark brown amorphous powder — was then siphoned from the bottom of the beaker, centrifuged, the water decanted off, and then dried at room temperature.

Exactly one gram of material was weighed into each of 24 two-inch petri dishes and these were divided into three series, each consisting of 8 dishes.

Series A was maintained at 4.5°C in a refrigerator

Series B ,, 21°C in an incubator

Series C ,, 34°C in a water bath

"B1" horizon material: The material was obtained from a well-developed podsol on Bagshot Sands bearing a heath vegetation dominated by *Deschampsia flexuosa* and *Calluna vulgaris*.

The black compacted sand was air dried and crumbled and the few visible roots removed. It was then lightly ground in a pestle and mortar and sieved through a very fine mesh sieve (90 gauge) giving a fine blackish powder which showed a loss on ignition of 15.6%.

Exactly three grams of this powder were weighed into each of 24 two-inch petri dishes and these were divided into three series each consisting of 8 dishes, as in the case of the "H" layer material.

"H" layer and "B" horizon material. Each series of 8 dishes were subjected to four different conditions, i.e.

- (1) maintained at optimum moisture conditions and fertilised with a mixture of inorganic salts (Mitchell 1934)
- (2) maintained at optimum moisture conditions and not fertilised
- (3) maintained at moisture conditions less than optimum and fertilised
- (4) maintained at moisture conditions less than optimum and not fertilised

Each sample was inoculated with one drop of a soil inoculum suspension †).

The "B" horizon material was ready on 25th January and the "H" layer material on 2nd February. The experiment was terminated on 8th June. After 3 months each dish was reinoculated with a further drop of soil inoculum †). The dishes intended to be maintained at optimum moisture conditions were kept in a soggy condition known empirically from experiment 2 to be approximately optimum. Those to be maintained at conditions less than optimum were somewhat arbitrarily given half the amount of water that the other dishes received. All the dishes were watered every second or third day.

Before considering the results it is necessary to draw attention to some of the limitations inherent in this experiment.

(1) The experiment ran for only four and a half months. This is less of a disadvantage than it would be in the case of an experiment on litter, since there is little seasonal fluctuation in the type of material present in the "H" layer and "B" horizon.

(2) The losses may be due partly to root hairs, root cells, animal cells and excretory products. The method of preparation of the material should have ensured however that such extraneous matter was present only in very small quantities.

(3) The results are not strictly comparable since the "B" horizon material was exposed to decomposition for one week longer than the "H" layer humus.

(c) *Results of Experiment 3.*

A summary both of the mean percentage losses in weight and of an analysis of variance is given below.

"H" layer material

Mean percentage loss in wt. per specimen	= 2.9%
" " " " " fertilised specimen	= 3.0%
" " " " " unfertilised specimen	= 2.7%
" " " " " watered specimen	= 3.0%
" " " " " $\frac{1}{2}$ watered specimen	= 2.7%
" " " " " specimen at 45°C	= 1.9%
" " " " " " " 21°C	= 2.5%
" " " " " " " 34°C	= 4.1%

†) See page 350.

*) This duplicated pair of results is purely coincidental

Summary of Analysis of Variance

Factors	F	Significance
Temperature	22.32	> 1% (highly significant)
Humidity	not significant	
Fertiliser	not significant	
Temp. & Humidity	12.95	> 1% (highly significant)
Temp. & Fertiliser	2.29	> 20% (doubtfully significant)
Humidity & Fertiliser	22.78	> 1% (highly significant)
Temp. & Humidity & Fertiliser . .	not significant	

"B" horizon material

Mean percentage loss in wt. per specimen	= 3.7%
" " " " " " fertilised specimen	= 3.5%
" " " " " " unfertilised specimen	= 4.0%
" " " " " " watered specimen	= 4.3%
" " " " " " unwatered specimen	= 3.1%
" " " " " " specimen at 45°C	= 2.2%
" " " " " " " " 21°C	= 3.6%
" " " " " " " " 34°C	= 5.4%

Summary of Analysis of Variance

Factor	F	Significance
Temperature	4.6	> 5% (significant)
Humidity	not significant	
Fertiliser	"	
Temp. & Humidity	4.2	> 5% (significant)
Temp. & Fertiliser	not significant	
Humidity & Fertiliser	"	
Temp., Humidity & Fertiliser	"	

The relatively inert character of both materials is apparent from these results. The mean loss in weight in the one case is 2.9% and in the other is 3.7% after a period of about 4 months *).

Fertilisation has had no appreciable effect upon the "B" material, whereas the Analysis of Variance carried out upon the "H" material shows the interaction between moisture and fertiliser to have been highly significant and that between temperature and fertiliser doubtfully significant.

Of the purely physical factors, moisture is in both cases without

*) Compare with the percentage loss in wt. of the litters which averaged about 45% after a period of 10 months.

significance, whereas the interaction of temperature and moisture is highly significant in the case of the "H" material and somewhat less significant in the case of the "B" material. This can be simply taken to mean that water may be a limiting factor in both organic and inorganic reactions. Increase in temperature has been accompanied in both cases by an increased rate of decomposition the significance of this being somewhat greater in the case of the "H" layer than of the "B" horizon material.

The general impression to be derived from these results is that the "B" horizon material, despite its greater absolute weight loss, is the more intractable material. Its rate of breakdown is uninfluenced by the addition of fertilisers while the relative effect of physical factors is appreciably less than is the case with "H" layer humus.

A small part of the greater absolute decomposition in the "B" humus may be explained by the additional two weeks of decomposition, this however is a relatively unimportant factor. It may be that the more purely colloidal condition of the humus and possibly the surface catalytic effect of the sand grains upon which the humus is deposited have resulted in a greater chemical activity than is possible in the "H" layer humus. It is, however, unlikely that the absolute difference in weight loss between the "B" and "H" humus is large enough to be of any significance.

DISCUSSION

(1) *Experiment 1:*

The few published results of experiments designed to investigate the mutual effects, upon rate of breakdown, of different forest litters, e.g. Hwang⁶⁾ 1938, suggest that the mixture of a potential mor-forming and a potential mull-forming litter decomposes — in a purely quantitative sense — more rapidly than either component. But it so happens that in these published experiments the mull-forming constituent has invariably been oak or beech litter. And it further happens that these two species, amongst all the temperate hardwoods, are probably the two most likely to form a raw humus. The present results have, in a rather curious fashion, tended to confirm these earlier findings. Whereas the admixture of a very good litter ("good" in the sense of strongly

mull-forming even on base poor soils) with Spruce has resulted in no synergistic effect, that of a moderate or poor litter, e.g. Red Oak or *P. radiata*, has in fact suggested that the two litters react mutually so that a mixture of the two decomposes more rapidly than either constituent. But the increase in rate of decomposition is not large.

	1st Species	% age loss	2nd Species	% age loss	Mixture	% age loss
Expt. 1..	Spruce	39%	Red Oak	37 %	50/50	44%
Expt. 2..	Spruce	37%	Red Oak	33.5%	50/50	44%
Expt. 1..	Spruce	39%	<i>P. radiata</i>	30 %	60/40	45%

The experiment does supply a partial answer to the question posed in the Introduction: "Does the rate of litter decomposition determine the type of humus produced?"

The straight line relationships established for the decomposition rates of Spruce — Hornbeam, Spruce — *Epilobium*, and Spruce — Hazel mixtures show that the broad-leaved litter has had no effect upon the rate of breakdown of the Spruce needles and yet it is reasonable to assume that the addition of the broad leaved litter would in fact have resulted in the formation of a mull type of humus.

A number of investigators such as Melin⁸⁾ and Broadfoot and Pierré⁹⁾ have attempted to correlate rate of decomposition with mull-forming ability. In this case Hornbeam having lost 56%, by weight in 10 months would be considered a silviculturally desirable litter while Hazel 43%, and Spruce 39% might be linked together as unfavourable to the soil. Such a proposition does not agree with field observation in general. Under natural conditions Hazel litter would disappear as quickly as Hornbeam and would be no less likely to produce mull. Bearing in mind the relatively large differences in rate of decomposition between the four hardwood species investigated, all of which with the possible exception of Red Oak are strongly mull-forming,

Percentage loss in 10 months

Red Oak		36%
Hornbeam		56%
<i>Epilobium</i>		53%
Hazel		43%

it is not logical to suggest that the increased weight loss brought

about by mixing together, e.g. Spruce and Pine represents a greater tendency to form mull rather than mor. It may be that this is the case but there is no evidence for it in these results.

The results of this experiment may be summarised by suggesting a tentative hypothesis, namely that the rate of decomposition does not appear to be a fundamental factor in mull or mor formation; but that while both mull and mor may be formed either quickly or slowly, depending upon the environment, there is the tendency, under comparable conditions for mull formation to be accompanied by a somewhat more rapid loss in weight of the litter undergoing decomposition.

No explanation is offered as to why two litters having mor-forming tendencies appear to react mutually leading to an increased rate of decomposition, while a characteristic mull-forming and a characteristic mor-forming litter have no quantitative effect one upon the other.

(2) *Experiment 2*

Red Oak was perhaps not the best choice for this experiment. A more contrasting litter might have been more interesting.

The results help to confirm the possibility that if two litters are mixed together, neither of which is strongly mull-forming, the mixture will lose weight more rapidly than would the pure constituents under similar conditions. But perhaps the most important result of this experiment is to draw attention to some of the errors inherent in earlier work. Thus the inversion effect suggests that the time of year at which the litter is collected, the length of time and the period of the year during which decomposition or loss in weight is measured may all have an important effect not only upon the absolute weight losses of different species but also upon their relative weight losses.

(3) *Experiment 3*

Mork⁹⁾ in 1938 investigated the rate of CO₂ evolution from humus and found that from easily nitrified humus the rate of CO₂ evolution rises with temperature at least up to 30°C whereas the more intractable the material the lower becomes the optimum temperature.

The results of experiment 3 disagree with Mork's⁹⁾ findings

in that a positive correlation exists between weight loss and temperature up to 34°C. But Mork⁹⁾ also suggested that the ratio $\frac{\text{CO}_2 \text{ evolution at } 30^\circ\text{C}}{\text{CO}_2 \text{ evolution at } 20^\circ\text{C}}$ might be used as a criterion of the quality of humus, the more easily nitrified the humus the higher being the ratio. In this, the present results may be interpreted as agreeing with those of Mork since the ratio $\frac{\text{loss in wt. at } 34^\circ\text{C}}{\text{loss in wt. at } 21^\circ\text{C}}$ was in the case of the "H" layer material 1.65 and in the case of the "B" horizon material 1.5.

A weakness of this, as indeed of all the experiments is that quantitative changes only have been measured. But it seems probable that profound changes in chemical composition would have been accompanied by greater weight losses than in fact have occurred with this material. It is therefore difficult to believe that the physical factors of the environment such as moisture, temperature and light, improved as a result of heavy thinnings, could alone induce large scale changes in humus quality, and since the organic matter of the 'B' horizon probably represents in a relatively pure form the ultimate "fixed protein" of Handley⁴⁾ it is difficult to believe that artificial fertilisation of forest sites can result in a widespread regeneration of such humus.

(4) General Conclusions

This final discussion is largely hypothetical and it is not believed that the results obtained from the experiments *in vitro* are necessarily applicable to natural conditions.

If it is assumed that an admixture of good hardwood litter such as Hazel does not alter the rate of decomposition of Spruce needles it must be at least possible that it has no effect upon the type of decomposition either, at least not in the first instance. But it is probable that such a mixture, provided the proportion of Hazel were sufficiently large, would either maintain a mull humus or, if added periodically to an existing raw humus, gradually give rise to a mull. It seems possible therefore that the hardwood litter acts not so much upon the existing raw humus as upon the site, gradually improving the soil conditions and encouraging a different soil fauna and flora. Thus if a regular supply of mull-forming litter began to fall upon a raw humus the new litter would supply a substrate

for more exacting soil organisms, and in proportion to its relative abundance would begin to alter the environment. In time, provided the supply continued, an important new factor might appear, namely the larger soil organisms such as worms, millepedes and even voles and other mammals. At this stage the litter and humus previously lying on the surface would become incorporated into the mineral soil. It is difficult to find evidence, that even at this stage, the litter previously producing mor would now produce mull. It is possible that the mor-forming fraction continues to produce mor when in mixture but that the raw humus so produced is, so to speak, carried along with the mull and distributed through the profile. On the other hand since pure Spruce probably continues to form a mull on base rich sites for an indefinite period it is likely that improved environmental conditions will be reflected in a qualitative change in decomposition. The evidence obtained from the experiments does at any rate suggest that the existing "B" horizon humus and to a certain extent the "H" layer material would remain relatively unchanged in character or at least that any change would be very slow.

SUMMARY

1. The evolution of ideas on raw humus formation is briefly reviewed.
2. It is shown experimentally that mixtures of the litters of Spruce and Hazel, Spruce and Hornbeam, and Spruce and *Epilobium angustifolium*, have no mutual effect so far as rate of decomposition *) is concerned.
- 3) Some evidence is provided that mixtures of the litters of Spruce and Red Oak and Spruce and *Pinus radiata* decompose more rapidly than either constituent alone.
- 4) A new technique of carrying out periodic determinations of oxygen absorption on living material is described.
- 5) The progress of decomposition of the litters of Spruce, Red Oak, and various mixtures of Spruce and Red Oak, was compared for the period of a year. It is shown that whereas Spruce litter respired more rapidly at the beginning of the period this tendency is reversed after about eight months.
- 6) The effects of moisture, temperature and inorganic nutrients upon the rate of decomposition of "B" horizon and "H" layer humus were investigated. It is shown that in all cases the absolute effects are small. In the case of "H" layer material, temperature, temperature $\times$ humidity

*) "decomposition" here and elsewhere throughout the paper is used in a sense synonymous with "loss in weight".

and humidity $\times$ fertilizer are all highly significant. In the case of "B" horizon material, temperature and temperature $\times$ humidity are significant.

7) It is suggested that the rate of decomposition is not a fundamental factor in mull or mor formation.

8) A tentative deduction is made that if qualitative changes are to be wrought in raw humus they must be initiated in the litter stages since the later humic stages are relatively intractable.

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THE VOLATILE FATTY ACIDS PRESENT IN FRESH AND IN FERMENTED MARROW-STEM KALE

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INTRODUCTION

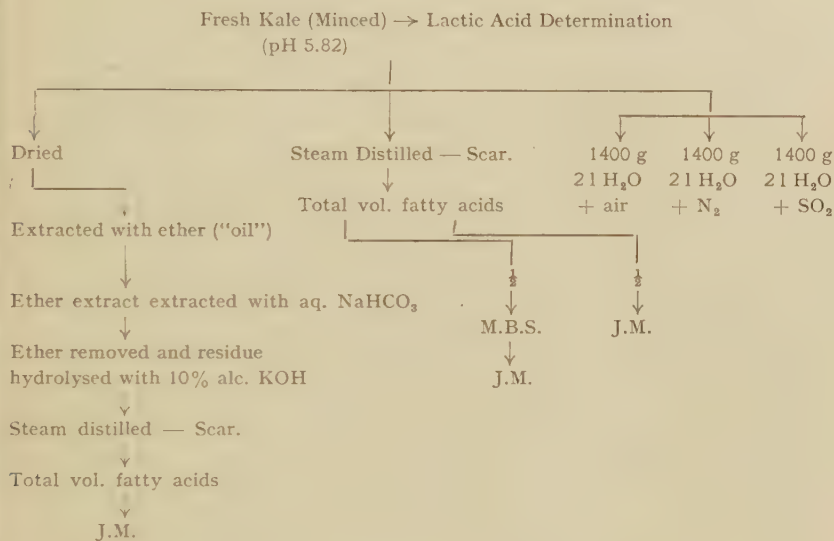
During the course of an investigation by Barnett and Duncan³⁾ into the fermentation of minced grass/water mixtures, it was noted that small quantities of acetic acid were present in the mixtures before it seemed possible that they could have been produced by microbial action. Experiments were then conducted on large amounts of fresh grass which were minced and steam distilled according to the method of Scarisbrick⁸⁾ within 20 minutes of being harvested. Examination of the distillate by the method of James and Martin⁴⁾ showed that only acetic acid was present and that to the extent of 8 mg% of the dry matter equivalent of the grass. Moreover, in these and parallel experiments no lactic acid (or acetaldehyde) was found to be present in fresh grass.

An examination has now been made of the volatile fatty acids in fresh minced marrow-stem kale (*Brassica Oleracea*), in the ether extract fraction of dried kale and in fermentation mixtures of minced kale and water.

EXPERIMENTAL

As stated above, the problem involved was a three sided one and the general scheme of attack is indicated in Fig. 1. The various techniques — the majority of which have already been described — are indicated below. The kale used in all the experiments actually described, was obtained from the same gross sample of the crop brought to the laboratory and it had a dry matter content of 16.00%.

Repetition of these experiments with other kale crop samples gave similar results.



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Fig. 1.

Volatile fatty acids in fresh kale

Fresh kale (100 g) was minced within 30 mins of cutting and steam distilled with 100 ml of water containing 10 g of potassium oxalate and 5 g of oxalic acid, to which solution had been added 5 ml of a 50% (V/V) solution of liquid paraffin in octyl alcohol. The distillate (2.5 l) was concentrated after neutralisation with NaOH to about 10 ml, reacidified and steam distilled in a Markham micro-Kjeldahl apparatus according to the directions of Scarisbrick (l.c.). The volume of distillate collected was 500 ml and of this 35 ml was analysed on a James and Martin (l.c.) column. A further 400 ml of distillate, after evaporation, was put through a Moyle, Baldwin and Scarisbrick⁵⁾ Type I buffered column and the material eluted with 1% (V/V) and 10% (V/V) butanol in chloroform. The eluate, free from the acetic acid

fraction, was then worked up in the usual way and run on the James & Martin column.

Volatile fatty acids present in combination in the ether extract of dried kale

Of the dried kale, 85 g were extracted with ether (redistilled) in a Soxhlet apparatus for 8 hrs. The ether extract was shaken (3 times) with saturated NaHCO_3 solution to remove any free acids. The separated ether layer was then dried over pure anhydrous Na_2SO_4 and after decantation, the ether was removed by evaporation. The residue was heated at the boiling point for 4 hrs with 10 ml of 7.5% (W/V) alcoholic KOH. The alcohol used had been freshly refluxed over lime and redistilled to remove any adventitious free acids. The hydrolysate was neutralised with 50% H_2SO_4 and half of it steam distilled, the distillate being subjected to gas-partition chromatography as described above.

The experiment was repeated using a petroleum ether (40° – 60°) solution of the extract and a column of alumina to remove chlorophyll and eluting with 2% (V/V) acetone in petroleum ether. The hydrolysis and distillations were then carried out as described with similar results.

Fermentation apparatus

The apparatus used in this work, and which has been used for a number of different types of laboratory experiments has been fully described by Barnett¹).

In the present instance, three aspirators were set up, each containing 1400 g of minced kale in admixture with 2 l of water. The first aspirator (A) was kept aerated by passing in a continuous stream of sterile air, the second (N) was kept under anaerobic conditions by passing in CO_2 - and O_2 -free sterile N_2 . In the case of the third aspirator (S) the 2 l of water contained 6 g of SO_2 . The fermentation reactions were allowed to proceed at room temperature for four weeks with daily analysis of the mixture to begin with and less frequent analysis later on.

Volatile fatty acids in fermentation mixtures of minced kale and water

Samples of effluent were withdrawn from slurries in the aspirators and preliminary separation of the volatile fatty acids was achieved

by steam distillation (Scarlsbrick; l.c.) of 10 ml portions of the initial samples. The volume of the samples used in later analysis was decreased to keep the total volatile fatty acids, per portion distilled, at the most convenient level which was equivalent to 2.4 ml of 0.02N NaOH. 80 ml of distillate was collected in every case and the volatile fatty acid content estimated by gas phase chromatography.

Lactic acid determinations

The determination of lactic acid on the fresh kale was performed according to the method of Barnett²⁾ for silage. Of the fresh kale, 10 g were heated to 60° with 100 ml of water and then stirred mechanically for 5 min. during which period 1 g of Ca(OH)₂ (Judex Brand) was added. Of the filtrate, 2 ml were then made up to 10 ml with water and of this dilution 1 ml. was used for the test by the method described.

In the case of the extracts from the slurry apparatus, analysis for lactic acid was made according to the method described by Barnett¹⁾.

RESULTS

In connection with the experimentation on fresh and fermented marrow-stem kale, the results are presented in Table I. It will be noted that in fresh kale, lactic, acetic and propionic acids are all

TABLE I

% Free Fatty Acids and Lactic Acid in Kale.															
% Fatty Acids and Lactic Acid formed during Fermentation of Kale.															
Time in days	Aerated Slurry					Anaerobic Slurry					Slurry + SO ₂				
	H.La	H.Ac	H.Pr	H.Bu	pH	H.La	H.Ac	H.Pr	H.Bu	pH	H.La	H.Ac	H.Pr	H.Bu	pH
Start	0.054	0.074	0.008	0	5.84	0.054	0.074	0.008	0	5.84	0.054	0.074	0.008	0	3.70
1	—	0.058	0.016	0	5.60	—	0.074	0.016	0	5.56	—	0.042	0	0	3.70
2	3.60	0.095	0	0	4.87	0.80	0.132	0	0	5.20	0.26	0.042	0	0	3.73
3	—	1.170	0	0	4.40	—	0.730	0	0	4.10	—	0.042	0	0	3.84
4	4.52	1.350	0	0	4.40	8.51	1.259	0	0	3.96	0.26	0.058	0	0	3.78
6	5.98	1.294	0	0	4.24	8.05	1.788	0	0	3.92	0	0.054	0	0	3.82
8	8.11	1.150	0	0	3.86	7.78	1.375	0	0	3.94	0	0.053	0	0	3.84
14	9.11	1.710	0	0.264	4.23	12.72	1.780	0	0	3.81	0	0.066	0	0	3.72
21	0.35	1.720	0.342	3.140	5.22	16.80	1.915	0	1.21	3.71	0	0.071	0	0	3.64
28	0	3.550	0.650	3.340	5.43	15.72	2.498	0.068	1.55	3.90	0	0.082	0	0	3.80

initially present. It will be further seen that the propionic acid increases slightly in slurries (A) and (N) and then disappears, while it disappears immediately — or at any rate after 24 hours — in the case of slurry (S). There is little change in the acetic acid content in slurries (A) and (N) till it begins to rise rapidly after the second day. In slurry (S) there is a decline in acetic acid content followed by a slow rise. The lactic acid content which rises rapidly in (A) and (N) falls away to zero in (S) after the first few days. These facts are consistent with the expectation of a decrease in enzyme activity, consequent upon the death of the plant cells — the changes being more abrupt in the slurry (S) containing the SO_2 — and the subsequent rise in concentration of the various acids due to the activities of micro-organisms. The production of acetic acid in (S) is due probably to coliform activity, certain strains of these organisms being capable of adjustment to low pH. During this period the pH of the aerated and anaerobic slurries reaches its minimum but the mixture containing SO_2 hardly changes in pH at all.

After 2 weeks in the case of the aerated mixture, butyric acid appears as the result of the activities of clostridia and the pH rises. In this slurry it continues to rise, the lactic acid disappearing with the formation of more butyric acid and also propionic acid. These changes occur also in the anaerobic slurry but more slowly.

With regard to the ether extracted material, the following acids were identified in the hydrolysate, in the amounts stated. The quantities are calculated on the known dry matter content of the kale.

	%
Formic acid	2.75 mg
Acetic acid	8.07
Propionic acid	0.66
n-Butyric acid	0.78
n-Caproic acid	0.65

When equal weights of milled dried leaves and milled dry stems were separately extracted and worked up as detailed above, it was found that, although there was much more ether soluble material in the leaves than in the stems (3.78% as against 1.48%), the nature of the acids was the same and equivalent weights of each extract gave the same amounts of these acids.

DISCUSSION

From the foregoing work it would appear that kale contains free, or very loosely combined, lactic, acetic and propionic acids. This would appear to be consistent with the general theory of fat synthesis but the fact that the presence of fatty acids higher than propionic acid could not be demonstrated was unexpected. The occurrence of such higher acids in combination with the ether extract fraction suggests the possibility that lactic acid, with acetic and propionic acids, are primaries in fat synthesis but that the actual fat synthesis is completed in the parts of the plant associated with fat storage.

It is true that Russell⁷⁾ has isolated a number of fatty acids from silage but these are deamination products of amino acids. Thus we have found that a minced grass/water mixture, kept at 37° for many months and of high pH, contained many straight and branched chain fatty acids but no free amino acids — the absence of the latter being demonstrable by paper partition chromatography. In general our findings with silage are similar to those of Virtanen and Miettinen⁹⁾. Raper⁶⁾ showed that lactic acid when fused with NaOH, could be converted to lower fatty acids but there is no question of this acid having escaped removal during the extraction with NaHCO₃. Moreover, we found no evidence of a possible lactic ester in the ether extract. What is possibly of interest however is that the mixed fatty acids formed from lactic acid by Raper's treatment contain acetic acid in the greatest amount just as in the case of our findings with the ether extract hydrolysate. Perhaps it is conceivable that the plant, by some mild enzymatic change, can bring about an *in vivo* reaction which requires fusing with caustic alkali in *in vitro* conditions. Certainly, boiling lactic acid with 10% KOH for 4 hours does not result in the formation of any lower fatty acids.

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SUMMARY

1) Lactic acid, acetic acid and propionic acids have been found to be present in small amounts in fresh kale.

2) The formation of lower fatty acids and of lactic acid in fermenting kale has been studied, using the technique of gas-partition chromatography, under different conditions, viz:- in aerated, anaerobic and partially sterilized mixtures.

3) A number of lower fatty acids have been shown to be present in the ethereal extract obtainable from dried kale.

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QUICK ANALYSIS OF SMALL SAMPLES OF NEW POTATO VARIETIES

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A good deal of work is done in the Netherlands on the breeding of new potato varieties suitable for starch processing and for stock feeding. For both purposes estimates of their content of dry matter are required. A potato for the starch factory should have a high starch content, whereas for stock feeding the crude protein content is of paramount importance.

For a first selection from many thousands of new seedlings ordinary laboratory methods are rather expensive. Tests are required which are cheap, simple, rapid and which can be adapted to make estimates in great numbers, where no great accuracy is needed. Our station has organized this work in such a way that a daily output of at least 50 samples is obtained by a small group of unskilled workers, with part-time supervision by one skilled analytical chemist.

PREPARATION OF THE SAMPLE

As a rule the separately harvested yield of 5-10 stalks is used for the analysis. The selection of a sample by hand from a bigger lot is not recommended for large scale work because of the enormous variability of the tubers of one variety, and even of one stalk⁵⁾. In exceptional cases the tests have been carried out with one tuber of 15-50 grams only. This however does not give a satisfactory evaluation of varietal properties.

An important point in every potato analysis is the use of a good desintegrator. A small scale saw blade mill is used of the same type as a starch factory mill (figure 1). A drum of 40 cm diameter and 18 cm width is coupled with an electromotor of $7\frac{1}{2}$ hp and 1440 rotations

per minute. A thin paste is obtained, which is evenly mixed before sampling.

DRY MATTER CONTENT

For most of the samples the breeder relies on specific gravity determinations. This property corresponds fairly well with the dry matter content, except for a small number of varieties which have "hollow heart" as a varietal property ⁶⁾.

A check is made on this point as soon as a variety has shown some chance of being of general use. In that case the dry matter content is determined by putting a spoonful of paste (5–10 grams) in a flat aluminium container of the *Meihuizen* apparatus ²⁾. If the sample selected is greater than 10 grams the container is overloaded. It must be rejected and another clean container used. The paste is dried first at about 50°C and when air-dry for one hour at 175°C.

STARCH CONTENT

Several good methods of starch determination are known ³⁾. They are however too intricate for execution by unskilled labour. Practised analysts are scarce and few of them have the perseverance to do the same type of analysis for weeks and months on end. We therefore avoid doing starch determinations except in limited numbers. An indirect method is described in the next paragraph.

DRY MATTER CONTENT IN PRESS JUICE

The dry matter content of the potato can be divided into three parts: starch, cell walls and the dry matter of the press juice.

The content of cell walls is about 1%. Its variation in the varieties used in the factory district is 0.1% only ⁸⁾. For breeding purposes it also can be considered constant. If therefore the magnitude of one of the other compounds is known, the second one can be calculated from the total dry matter content (or the specific gravity).

The dry matter content of the press juice is most easily determined, viz. by refractometer. Figure 2 gives the relation between refractive index and dry matter content for a number of samples from the Netherlands factory district. The correlation is not as good as would be expected for a solution of salts, sugars and proteins.

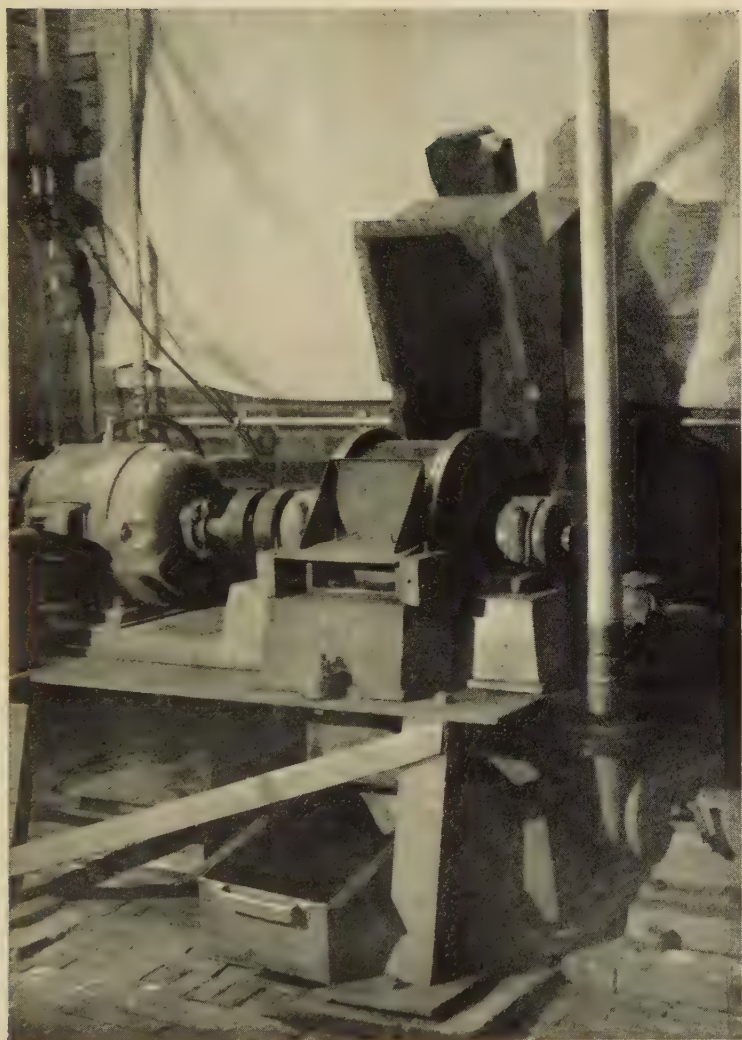


Fig. I. Saw blade desintegrator.

The juice contains a varying amount of insoluble matter (protein, tannins, coloured bodies) which does not influence the refractive index. It is also somewhat difficult to eliminate the smallest starch grains. Nevertheless the relation is sufficient to make an estimate of the starch content which is adequate for breeding purposes, by subtracting soluble dry matter from total dry matter as estimated by specific gravity.

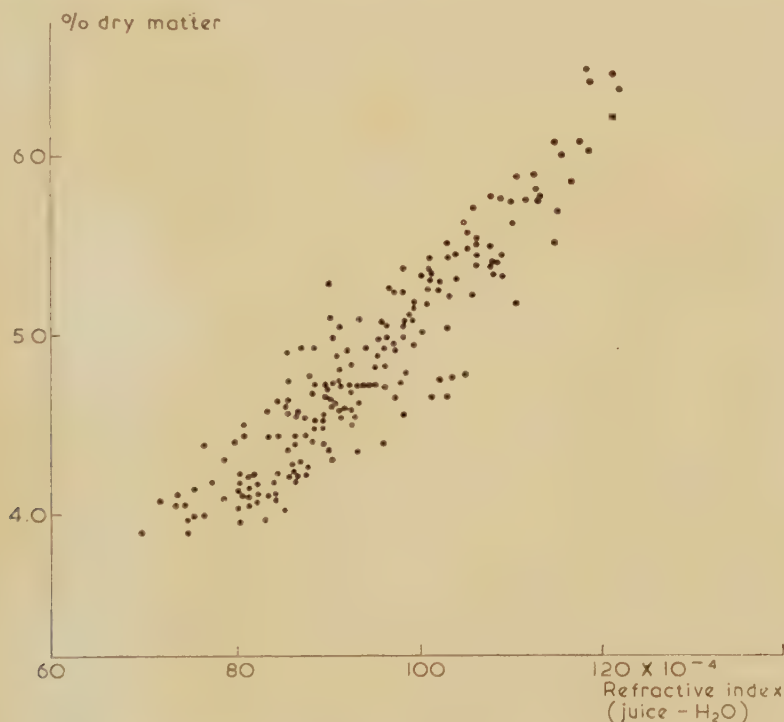


Fig. 2. Correlation between % dry matter and refractive index of potato press juice.

NITROGEN CONTENT

The direct Kjeldahl determination in the potato paste is hampered by foaming, caused mainly by the amount of water which must be evaporated before destruction begins. Addition of water should be avoided. Even without extra water the destruction needs incessant supervision.

It was found however that the destruction of the press juice goes

on with much less foaming and in a very short time; so that almost no supervision is necessary. Between this value and the total nitrogen content of the potato paste a fairly good correlation exists (figure 3). A still better agreement is found if the varying starch content of the samples is allowed for.

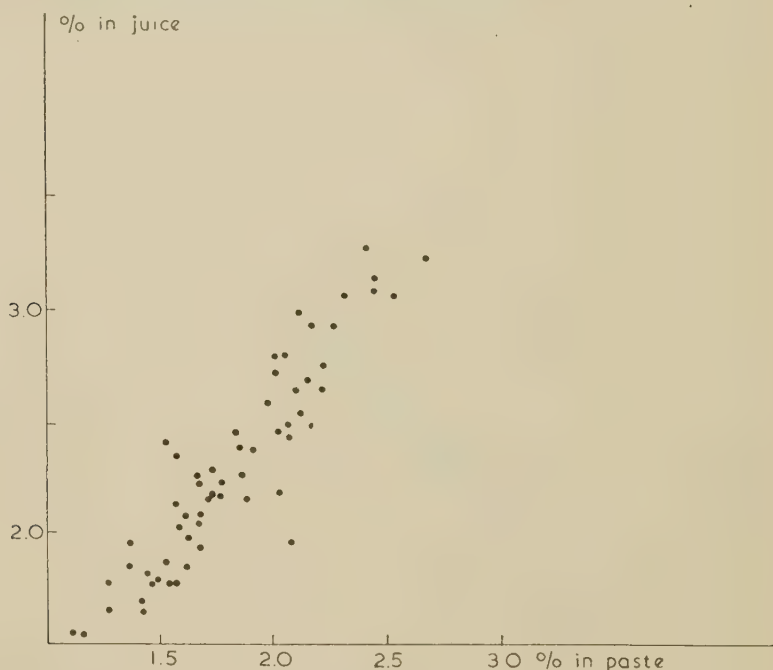


Fig. 3. Correlation between nitrogen content ($N \times 6.25$) as estimated in the whole paste and in the juice.

POTASSIUM CONTENT OF THE PRESS JUICE

An estimate of the potassium content is made by conductivity measurement ⁷). This value corresponds well with the potassium content and also with the total ash content, provided the chlorine content of the plant is not great.

The determination is very easy. It takes less than a minute if combined with the determination of refractive index and can be done by unskilled labour. Varietal differences are found, but they are relatively small with respect to the changes in potassium uptake caused by unequalities in the soil, and to the varietal differences in this uptake.

OTHER COMPONENTS OF THE PRESS JUICE

Potassium salts and nitrogen containing components form the major part of the dry matter of the juice, often more than 80%. Hence if the remainder could be supposed constant (viz. not fluctuating more than 0.1 or 0.2%) one of the determinations would be superfluous. In that case the Kjeldahl determination could be discarded, it being the most intricate one. A sufficient estimate of nitrogen content could then be made by the measurement of refractive index combined with that of conductivity.

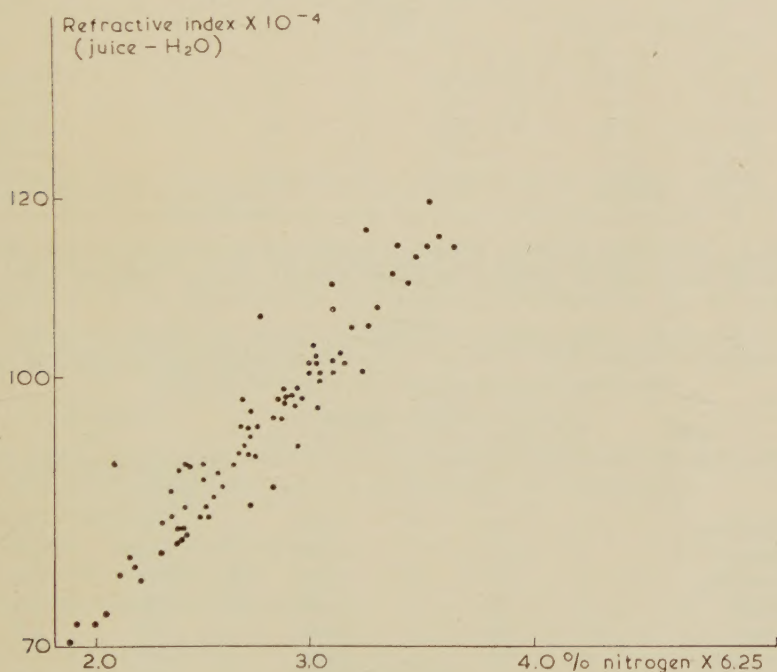


Fig. 4. Correlation between refractive index and nitrogen content of the juice after correction for different conductivity (variety Ultimus only).

This is the case with samples of one variety, provided the tubers are harvested and kept under such conditions that sugar formation is prevented. Figures 4 and 5 give an example relating to a group of samples of the variety Ultimus, grown on sand peat mixtures and harvested in September and October 1947, viz. before cold weather had set in. The original refractive index was supposed to be additively

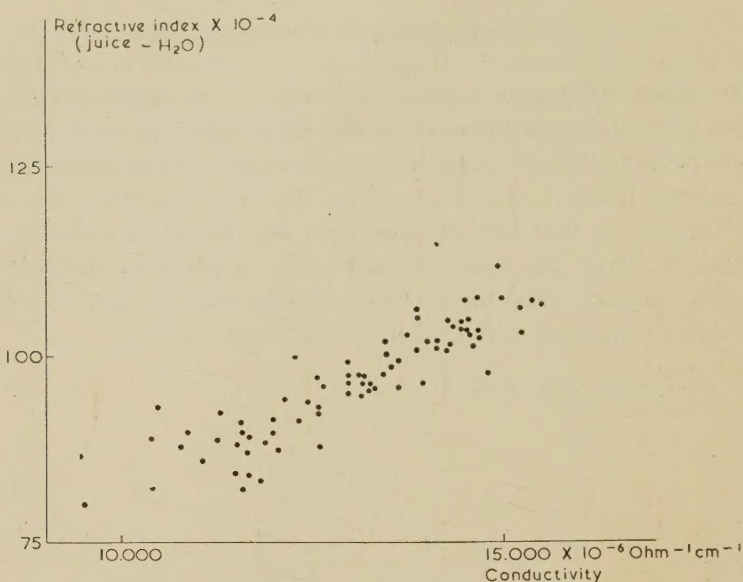


Fig. 5. Correlation between refractive index and conductivity of the juice after correction for different nitrogen content (variety Ultimus only).

TABLE I

Analysis of the press juice of a series of normal Netherlands varieties, cultivated in 1949. Mean values for samples from 4 experimental fields				
Variety	Refractive index increment $\times 10^4$	Conductivity $10^{-6} \text{ Ohm}^{-1} \text{ cm}^{-1}$	Nitrogen % $\times 6.25$	
			calculated from fig. 4—5	found
Noordeling	99	12700	3.18	3.50
Libertas	95	12700	3.04	3.19
Rode Star	93	13000	2.94	3.14
Record	85	12100	2.82	2.96
Voran	86	11600	2.94	2.94
Noordstar	82	12100	2.69	2.74
Thorma	80	10600	2.91	2.39
Sientje	77	11000	2.73	2.44

composed by the refractive indices of the potassium salt solution, of the solution of nitrogenous matter and of a third group of substances. The figures are the result of a polyfactor analysis ⁴⁾ in which corrections have been made for the first two factors. The variance in these figures is sufficiently small to be negligible for the purpose in discussion. In this case the influence of the third group of substances can therefore be disregarded.

However the relation between refractive index, nitrogen content and conductivity is not so simple in other varieties. Table I gives a case in which the estimate of the nitrogen content is at least right in a qualitative sense. But the first three varieties of table II have a higher estimate for nitrogen content than the last ones, although their real content is lower.

TABLE II

Analysis of the press juice of five potato varieties, cultivated in 1952. Mean values for 5 samples of experimental fields *)				
Variety	Refractive index increment $\times 10^4$	Conductivity $10^{-6} \text{ Ohm}^{-1} \text{ cm}^{-1}$	Nitrogen % $\times 6.25$	
			calculated from fig. 4—5	found
Capella	95	12200	3.02	2.80
Panther	93	12200	2.95	2.76
Fortuna	93	12000	2.99	2.88
Record.	90	11800	2.92	2.91
Voran	88	11400	2.92	2.92

*) Samples have been obtained from W.A. Scholten's Aardappelmeelfabrieken N.V.

The differences must be interpreted as the presence of a higher content of other constituents, probably carbohydrates. Up to now we have not found a cheap method for determining these constituents, suitable for serial work.

SUMMARY

An estimate of the starch, nitrogen, potassium and soluble carbohydrate content of the potato is obtained by the following determinations:

Specific gravity

Refractive index of the juice

Conductivity of the juice

Nitrogen content of the juice.

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